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INTRAVENOUS ACTH AND ORAL CORTISONE IN THE TREATMENT OF BRONCHIAL ASTHMA

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IN THE past two years many investigators have shown that ACTH and cortisone will relieve the symptoms of the majority of patients with bronchial asthma within 48 to 72 hours. However, this relief is only temporary; with cessation of therapy, symptoms usually recur within 4 to 6 weeks, occasionally within 24 hours and more rarely will recur as late as two hundred and eighty days after therapy. In view of the temporary nature of this remission (which is achieved at considerable expense), we have attempted to determine whether intravenous ACTH would be more effective, less costly, and provide a greater period of freedom than ACTH by the previously used routes.

Recently, Renold and associates,[1] and others[2, 3] reported that ACTH given intravenously by means of a slow, prolonged infusion was very effective in much smaller dosages than those employed by the intramuscular route. The purpose of this paper is to present our observations on the use of intravenous ACTH therapy in sereve intractable asthma or status asthmaticus, and also on the use of oral cortisone therapy for the maintenance of remission following such treatment.

INTRAVENOUS ACTH* THERAPY

Case Material.—Twenty-eight patients with severe intractable asthma or status asthmaticus were given ACTH intravenously at the Buffalo General Hospital or at the Children's Hospital. Five of these had two courses of this therapy. There were 16 females and 12 males ranging from 6 to 71 years of age. The duration of their bronchial asthma ranged from 4 months to 42 years. Sixteen had been ill for more than 10 years; only 6 had suffered from this disease for less than 5 years. Positive skin tests to many allergens were present in 9 of the 28 patients. Three had severe aspirin sensitivity. Seventeen had asso-

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*All ACTH furnished through the courtesy of Armour & Company. ACTHAR, lot #R521812 from sheep pituitaries used throughout this study.

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ciated emphysema or bronchiectasis or both. Three of these had definite cor pulmonale as determined by electrocardiograms and x-rays. Seven patients had other associated allergic disorders such as rhinitis, nasal polyps, or eczema.

Procedure.—All patients were carefully observed in the hospital for 3 to 5 days prior to the use of ACTH therapy. During this period the usual methods of therapy, such as aminophylline, epinephrine, ether in oil, etc., were employed. Only patients who failed to respond favorably to these medications were used in our study.

All patients were examined daily. In addition to the evaluation of the clinical response, the effect of the ACTH was observed on volume of sputum, body weight, blood pressure, the concentration of blood glucose, serum sodium, serum potassium, eosinophils, and electrocardiograms.

Dosage Schedule.—The ACTH powder was dissolved in 1,000 c.c. of 5 per cent glucose in distilled water and was administered by a slow continuous intravenous drip for periods varying from 8 to 12 hours. The veins in the forearm or wrist were frequently employed, so that the patient would have less restriction of motion. The initial dose was 20 mg. of ACTH daily for 2 to 3 days. The amount was gradually reduced over a period of 6 to 14 days and given in the same manner. The total dosage ranged from 75 to 220 mg. (Table I).

TABLE I. SCHEDULE OF INTRAVENOUS ACTH THERAPY IN BRONCHIAL ASTHMA

DAY	AMOUNT* (MG.)	DAY	AMOUNT* (MG.)
1	20	5	15
2	20	6	10
3	20	7	10
4	15	8	5
Total 115 mg.			

*Dissolved in 1,000 c.c. of 5 per cent glucose in water and administered over an 8 to 10 hour period.

All patients were placed on a low sodium diet and given supplemental potassium, either in the form of saturated solution of the iodide or as potassium chloride tablets.

RESULTS

Clinical Response.—A total of 33 courses of intravenous ACTH was given to 28 cases. Twenty-seven of these 33 courses produced symptomatic relief within 24 to 48 hours. However, improvement by objective signs was not evidenced, in most instances, until 72 to 96 hours after starting therapy. Except for the potassium iodide that was given to most patients, only 6 of the 28 patients and of the 33 courses required other medications during the intravenous course, such as the epinephrine nebulizer, epinephrine, or aminophylline.

Four of the 28 patients had, in addition to their asthma, severe nasal polyps and allergic rhinitis. All had improvement in their nasal symptoms, and a marked shrinkage of the size of the polyps. The bronchial symptoms, however, cleared more rapidly than did the polyps and nasal symptoms.

TABLE II. PERIOD OF REMISSION FOLLOWING INTRAVENOUS ACTH

WEEKS WELL	NUMBER OF COURSES
0	6
1-2	5
3-4	11
5-6	6
9	2
26-40	3

One other patient had an associated severe generalized atopic eczema which cleared up rapidly within four days. Upon cessation of ACTH therapy, however, the skin lesions returned within 24 hours, although the asthma remained in remission for 8 weeks.

Still another patient had relief of her osteoarthritis.

Period of Remission.—Twenty-two of the 33 courses of intravenous ACTH were followed by freedom of symptoms for three weeks or more (Table II). It is interesting to note that 12 of 17 courses in complicated cases, who had either emphysema, bronchiectasis, and/or cor pulmonale, were in this group.

Ten of the 16 courses in uncomplicated asthmatics were similarly benefited. Total ACTH dosage given varied from 75 to 220 mg. There did not appear to be any increased benefit or prolongation of the period of remission with an increase in the dosage. Likewise, the length of the period of treatment which varied from six to fifteen days had no effect on these results.

Physiological Changes (Table III).—

1. *Eosinophil response:* Twenty-six of the 27 patients studied for the phenomenon had a drop of 50 per cent or more in the total eosinophil count after the start of therapy. There did not appear to be a relationship between eosinopenia and relief of symptoms, as 5 of the patients who failed to show clinical improvement had a significant eosinophil response. The one patient who failed to show an eosinopenia had good clinical relief.

2. *Sputum volume:* This was decreased considerably in 11 cases, increased in 2, and was unchanged in the remainder.

3. *Edema:* As evidenced by a weight gain of more than 4 pounds, edema developed in 10 of these patients.

4. *Blood chemistry:* An increase in the serum sodium was noted in 5 cases and a decrease in serum potassium in 2. There was no change in the blood glucose in 10 patients so studied.

TABLE III. PHYSIOLOGIC CHANGES DURING TREATMENT

DAILY OBSERVATIONS		NUMBER OF CASES
Blood Pressure	No Change in	33
Weight Gain		10
Sputum Volume	Increased in	2
	Decreased in	11
PERIODIC OBSERVATIONS		5
Serum Potassium	Decreased in	26 of 27 studied
Serum Sodium	Increased in	
Eosinophil Response (Decreased 50% or more)		2

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5. *Miscellaneous findings:* There was no change noted during the course of therapy in the blood pressure,* urinalysis, x-rays of the chest,† or electrocardiograms in any of these patients.

6. *Pulmonary function studies‡:* Spirograms were performed according to the technique of Baldwin, Cournand, and Richards.[4] After two or more trials to make sure that the patient's best performance had been recorded a bronchodilator spray, Vaponefrin,§ was administered and a repeat spirogram recorded after 8 to 12 minutes. From the spirograms the vital capacity and maximum breathing capacity were calcu'ated and compared with the predicted normal figures determined from nomograms based on the formulas of Baldwin.[4]

In 9 patients spirograms were obtained shortly after hospital admission. Intravenous infusions of 5 per cent glucose in water were given to these patients for 3 to 5 days. They were under the impression that this was ACTH treatment. The spirogram was repeated, and then the infusions were continued but with ACTH added. The patients were unaware that any change in medication had been made. Immediately after ACTH therapy was completed a third spirographic observation was made. The results of the functional tests in this controlled group are shown in Table IV.

TABLE IV

	VITAL CAPACITY (C.C.)				MAXIMUM BREATHING CAPACITY (L/MIN.)			
PATIENT NO.	PREDICTED NORMAL*	BEFORE GLUCOSE	AFTER GLUCOSE, BEFORE ACTH	AFTER ACTH	PREDICTED NORMAL*	BEFORE GLUCOSE	AFTER GLUCOSE, BEFORE ACTH	AFTER ACTH
1	3700	4250	3900	4630	95	68	42	81
2	4140	3480	3120	3770	120	39	39	42
3	2300	1460	1330	2030	62	26	36	39
4	2900	2310	2030	2560	86	47	34	55
5	3380	1700	1490	2560	95	26	26	34
6	2520	1250	1430	2220	73	44	36	52
7	4350	1760	1520	2310	119	36	29	57
8	2580	785	1490	2080	69	21	26	60
9	3540	2200	2770	3900	112	42	42	91

*From nomograms based on the formulas of Baldwin, Cournand, and Richards, Medicine 27: 243, 1948.

The results are of interest in that every patient showed an increase in both vital capacity and maximum breathing capacity in the third test after ACTH treatment, over the values obtained in either of the first two tests. This increase was larger than 15 per cent over either the first or second test values in 5 of the 9 patients for both vital capacity and maximum breathing capacity increased less than 15 per cent in patients No. 2 and No. 3. The significance of the increase in pulmonary function with ACTH is enhanced by the fact that during the control period of glucose infusions only two patients showed an improvement in maximum breathing capacity, and only threee in vital capacity.

In 13 additional patients spirograms were obtained before and after treatment with ACTH but without regard to the institution of other treatment.

*Exception: Case: A. R. (See discussion).

†Exception: Case: A. M. (See discussion).

‡This work was supported in part by the Masonic Foundation for Medical Research and Human Welfare.

§Supplied through the courtesy of the Vaponefrin Co., Upper Darby, Pa.

TABLE V. EFFECT OF ACTH INTRAVENOUSLY ON MAXIMUM BREATHING CAPACITY

	TOTAL NUMBER OF PATIENTS	MBC INCREASED		MBC UNCHANGED	
		NUMBER OF PATIENTS	CLINICALLY IMPROVED	NUMBER OF PATIENTS	CLINICALLY IMPROVED
Asthmatics					
With Emphysema	12	6	5	6	3
Without Pulmonary Complications	10	6	5	4	2

The first spirogram was usually obtained after several days' observation in the hospital, then ACTH was given, and a second spirogram was done.

Although these 13 cases are not as adequately controlled as the first group of 9 described above, the following results, as summarized in Table V, were obtained in the total 22 courses.

Twelve patients in this group had either emphysema, pulmonary fibrosis, and/or cor pulmonale, or a combination of the three, associated with their asthma. There was no significant increase in the maximum breathing capacity in 6 of these 12 after ACTH therapy. Three of these 12 failed to have a good clinical response. One other patient, despite an increase in the maximum breathing capacity, failed to improve clinically following ACTH therapy.

Four of the remaining 10 patients without pulmonary complications did not show an increase in the maximum breathing capacity following this therapy. Two of these patients also had no clinical improvement. One patient, despite an increase in the maximum breathing capacity, had no decrease in symptoms after ACTH therapy.

Hence, as can be seen from these results, the failure of the maximum breathing capacity to increase did not necessarily indicate an unsatisfactory degree of clinical improvement. Contrariwise, an increase in the maximum breathing capacity was not always associated with a favorable clinical response.

Changes were noted in the vital capacity and maximum breathing capacity when a bronchodilator spray was given in this group of patients, both before and after ACTH treatment. The degree of response to ACTH treatment could not be predicted by the change effected by the bronchodilator spray.

SIDE EFFECTS

In addition to the exaggerated physiologic response described above, certain other effects were encountered. (Table VI.) Definite euphoria was noted in 17 patients. Two patients complained of epigastric pain while on ACTH. Both had a previous history of peptic ulcer. However, with the use of anti-

TABLE VI. SIDE EFFECTS OF INTRAVENOUS ACTH THERAPY

	NUMBER OF PATIENTS
Euphoria	17
Gastrointestinal distress	2
Masked infection	2
Urticaria	1
Phlebitis of arm veins	2

Results in 28 patients (33 courses)

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spasmodics and antacids, the therapy was continued. X-rays failed to show evidence of activation of the lesions. Two other patients had "masked" infections with no elevation of temperature or leukocyte counts while on ACTH therapy, but 24 hours later, after discontinuance of the ACTH, there was a marked rise in temperature and of the white count. Antibiotic therapy controlled these infections, one a pneumonia, the other a flare-up of a bronchiectasis. Phlebitis of the arm veins used for the intravenous infusion developed in two patients. Generalized urticaria was produced in one patient each time the ACTH was given. This was controlled by adding Benadryl to the solution and the ACTH was continued.

ORAL CORTISONE* THERAPY

Cortisone was given orally to three groups of patients, namely, those who had a recurrence of symptoms following intravenous ACTH therapy (Group A); those following intramuscular ACTH (Group B); and to ambulatory asthmatics not responding to the usual antiasthmatic measures without previous ACTH (Group C).

DOSAGE

All patients were given the same schedule of dosage the first week. The first day, a total of 300 mg. was administered in four equally divided doses. The second day, 200 mg., and from the third through the seventh day, 25 mg. four times daily. The dosage was then decreased gradually to the minimal amount which would adequately control the symptoms.

RESULTS

Group A (Following Intravenous ACTH Therapy).—Fifteen patients who had a recurrence of symptoms after their course of intravenous ACTH therapy received oral cortisone. Four of this group had an excellent response from the cortisone. (Table VII.) Two of these remained free of symptoms for several

TABLE VII. EFFECT OF CORTISONE ON BRONCHIAL ASTHMA

	NUMBER OF CASES	RESULTS			
		NONE	FAIR	GOOD*	EXCELLENT
Group A (Following remission after IV ACTH)	15	2	3	6	4
Group B (Following remission after IM ACTH)	7	2	1	2	2
Group C (Without previous ACTH)	13	3	3	3	4
Total	31	7	6	9	9

*Satisfactory relief, but considerable other antiasthmatic measures necessary.

weeks after omitting the cortisone. The other two patients had an immediate recurrence of asthma when the daily dosage of steroid was dropped below the maintenance level. Six additional patients had good results while on cortisone

*Cortisone supplied in part through the courtesy of F. Merck & Co., Rahway, N. J.

therapy, but still had to resort to other antiasthmatic measures for comfort. Five of the 15 patients in this group had to stop the cortisone because of marked weight gain.

Group B (Cortisone Following Intramuscular ACTH).—Oral cortisone was given to 6 patients whose symptoms recurred after intramuscularly administered ACTH (Table VII). One patient received two such courses of cortisone. Excellent results were obtained in two patients, one of whom remained well for over one year after stopping cortisone therapy. However, an allergic regimen was carried out while she was on the steroid therapy. Another patient, who responded fairly well at first, soon had a marked exacerbation of symptoms which could not be controlled by as much as 200 mg. to 300 mg. of cortisone daily. Two other patients had good responses, but needed considerable other antiasthmatic measures for sufficient symptomatic relief, and the final two patients were not benefited whatsoever. One patient who received two separate courses of therapy had an excellent response to the first course, but no help with the subsequent course. Only one patient of this group had to stop treatment because of weight gain.

Group C (Cortisone Without Previous ACTH Therapy).—Cortisone was prescribed to 13 asthmatics whose symptoms were not adequately controlled by the usual medications and treatment. Seven had a good or excellent response while on this therapy. One of this group had relief of symptoms for 12 weeks after stopping cortisone but, here again, specific allergic management had been reinstituted. Two patients, both with emphysema, claimed that their symptoms were made worse on cortisone therapy.

MAINTENANCE THERAPY

Only those patients of the above three groups who had either good or excellent responses to cortisone therapy were given continued dosage. The daily amount of cortisone was gradually reduced every three or four days to the minimal amount which would control the symptoms. This varied considerably for different patients. Some patients could gradually stop the drug and be without it several weeks at a time. Others required from 50 to 100 mg. daily to keep them comfortable and still others as little as 12.5 mg. every other day. However, the mean daily maintenance dose was 50 to 75 mg.

DURATION OF THERAPY (TABLE VIII)

Four patients have received cortisone for more than seven months, two of these over one year. An additional six have been maintained on cortisone for four to six months.

SIDE EFFECTS

The most prevalent of all side effects noted was that of water retention or marked weight gain (Table IX). This appeared more frequently in the group who received cortisone after either intravenous or intramuscular ACTH. Ten of 21 patients so treated had marked weight gain, whereas only 3 of 13 patients who received cortisone without previous ACTH were so effected.

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TABLE VIII. DURATION OF MAINTENANCE THERAPY WITH ORAL CORTISONE

LENGTH OF TIME (MONTHS)	NUMBER OF PATIENTS
7-14 months	4
5- 6 months	2
3- 4 months	4
1- 2 months	11
1 month or less	15

A 6-year-old boy, usually very quiet, became emotionally disturbed and almost maniacal while on the steroid. Two patients developed a moon face, and blurring of vision was claimed by another. Urticaria of the feet developed each time one woman took cortisone. Almost all the patients had a sense of well-being. There was no evidence of increased blood pressure or glycosuria in any of our patients, either during the initial course of decreasing doses or on the minimal maintenance dose.

DISCUSSION

From the results of the two series of patients treated with intravenous ACTH, it is apparent that this method is satisfactory. Improvement was noted, which was comparable to that seen in similar series treated with intramuscular ACTH.[5-8] This improvement, similar to that obtained by the intramuscular route, is temporary, and does not persist after treatment was discontinued. The usual physiologic responses to hormonal therapy were observed, and the side effects were no more disturbing or more frequent than those reported from intramuscular administration. The great advantage of this method of treatment is the economy of material. The results obtained with 100 to 125 mg. were comparable to those seen with 800 to 1,200 mg. by intramuscular means.[5-8]

Also, the intravenous route, at times, can be effective when there is no clinical response to its use intramuscularly. One such case presented itself in our series. A. M., a 12-year-old girl with periarteritis nodosa, bronchial asthma, and transitory infiltration in the lungs, had responded to three previous courses of intramuscular ACTH. In each instance there was a drop in eosinophils and clearing of the lungs. However, with the fourth course of intramuscular ACTH in dosages up to 40 mg. every six hours for several days, no eosinophilic response or clinical improvement developed. An intravenous infusion of 20 mg. of

TABLE IX. SIDE EFFECTS OF CORTISONE

	GROUP A AFTER IV ACTH	GROUP B AFTER IM ACTH	GROUP C WITHOUT PREVIOUS ACTH
Number of patients	15	6	13
Weight gain	6	4	3
Moon face	1	2	
Blurring of vision		1	
Emotional disturbance			1
Urticaria	1		
Aggravation of respiratory symptoms			2

ACTH was instituted and within 12 hours (before the infusion was completed) there was a dramatic clinical improvement and eosinopenia, followed by x-ray evidence of complete clearing of the lungs.

The disadvantages of this method of steroid therapy are that hospitalization is necessary, there is some immobilization of the patient and there is the possibility of a more severe allergic reaction from material used intravenously.

Why some patients respond and others do not has not been answered by our experiences. It has been postulated that the best responses are seen in patients with uncomplicated asthma, and that hormonal therapy may be contraindicated in emphysema, bronchiectasis, and cor pulmonale.[9] This has not been the case in our series, where the response to intravenous ACTH has been about the same in the two groups. However, two of our "complicated" asthmatics did claim that they were made worse by oral cortisone.

The mechanism of action of ACTH in the relief of bronchial asthma is not yet clearly established. The beneficial effects are probably mediated through some other mechanism than that through which nebulized bronchodilator (epinephrine) is effective. These patients responded in varying degrees to such a spray and the lack of correlation with the response to ACTH treatment suggests that their action is different. It is known that the action is not at the antigen antibody level, but probably a direct action on the reactivity of the bronchial cells.

The above studies indicate that certain patients can be kept fairly comfortable for many months by oral cortisone therapy. Likewise, others[10, 11] have reported that ACTH in small intramuscular doses can produce similar effects. However, little is known of the effect of prolonged usage of these steroids on the entire endocrine system. Will too much substitution therapy with cortisone produce atrophy of the adrenals or will overstimulation with ACTH produce hypertrophy? It has been suggested that alternate courses of ACTH and cortisone be given as maintenance therapy. Although oral cortisone has proved a simple method to administer for such prolonged treatment, we have shown that following ACTH therapy approximately 50 per cent of the patients develop definite edema from cortisone. We are now employing ACTHAR Gel* for maintenance treatment after intravenous ACTH, in an attempt to overcome this difficulty.

One must be fully cognizant of the contraindications in the use of these steroids. When necessary, ACTH and cortisone can be given with extreme caution, to certain individuals, who, ordinarily, should not receive these substances. With these limitations in mind, two patients in our series with severe hypertension were given ACTH. The remarkable finding was that one of these patients, A. R., whose blood pressure prior to therapy was 260/140, showed a gradual return to normal values while receiving ACTH intravenously. Previous hospital admissions without ACTH therapy failed to reduce the hypertension.

ACTH and cortisone therapy is not proposed as a substitute for specific diagnosis and treatment, but rather as a means of aiding the patient during a period of great stress, such as status asthmaticus or a severe acute allergic reaction such as a drug sensitivity reaction.

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SUMMARY AND CONCLUSION

ACTH intravenously is equally as effective as intramuscular ACTH in the treatment of severe bronchial asthma and the dosage required is much smaller. Twenty-seven of 33 patients obtained temporary relief. The average length of remission was three to four weeks.

Six patients with intractable asthma were kept comfortable by taking oral cortisone over a period of 6 to 14 months. Likewise, cortisone gave satisfactory temporary relief to 18 of 31 ambulatory asthmatics.

We wish to thank Miss Shirlee M. Kemp for her technical assistance with the pulmonary function studies.

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EFFECT OF CORTISONE UPON THE RISE OF SERUM ANTIBODY LEVEL AFTER THE INJECTION OF A SATURATING DOSE OF ANTIGEN IN RABBITS SENSITIZED TO OVALBUMIN*

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THE remarkable clinical results obtained with ACTH and cortisone in the treatment of allergic conditions such as allergic rhinitis, drug allergy, allergic dermatitis, and allergic asthma[1-8] gave rise to the hypothesis that these hormones could interfere with some fundamental mechanism of the allergic state, such as the antigen-antibody reaction. Recent published data, however, have shown that these hormones cannot modify a state of acquired anaphylactic sensitivity whether it has been actively or passively acquired. They cannot protect the animal against anaphylaxis[9, 10] or the Arthus reaction.[11] They cannot inhibit the Prausnitz-Küstner reaction in man.[12] These hormones, nevertheless, can exert a definite influence upon antibody production during the sensitization period. The results published by Germuth and Ottinger,[13] who used ovalbumin as an antigen, and those reported by Bjorneboe, Fischel, and Stoerk,[14] who use polyvalent pneumococcic vaccine, have shown that if ACTH or cortisone are injected during the sensitization period, the antibody level obtained in the serum was considerably smaller in the treated animals, than in the control animals.

It is difficult to understand how these hormones modify the production of antibodies. It is known that cortisone[15] can inhibit the activity of tissues from which the cells which are believed to produce the antibodies are derived, e.g., the reticulo-endothelial tissues, the plasmocytes,[16] or the lymphocytes.[17, 18] It is probable that these hormones inhibit antibody synthesis by the cells themselves. On the other hand, Dougherty, Chase, and White[19, 20] have shown that the injection of corticotropic hormones and of hormones of the adrenal cortex can cause a certain increase of the antibody level of the blood in the immunized animal as well as an important decrease in the number of lymphocytes in the lymphatic tissues. These two sets of data seem to be contradictory and need a logical explanation.

An objection can be raised to the experiments which require a prolonged administration of hormones during the period of sensitization. It is well known that treatment over an extended period of time with these hormones, as the experiments require, usually causes a deterioration of the physiological balance

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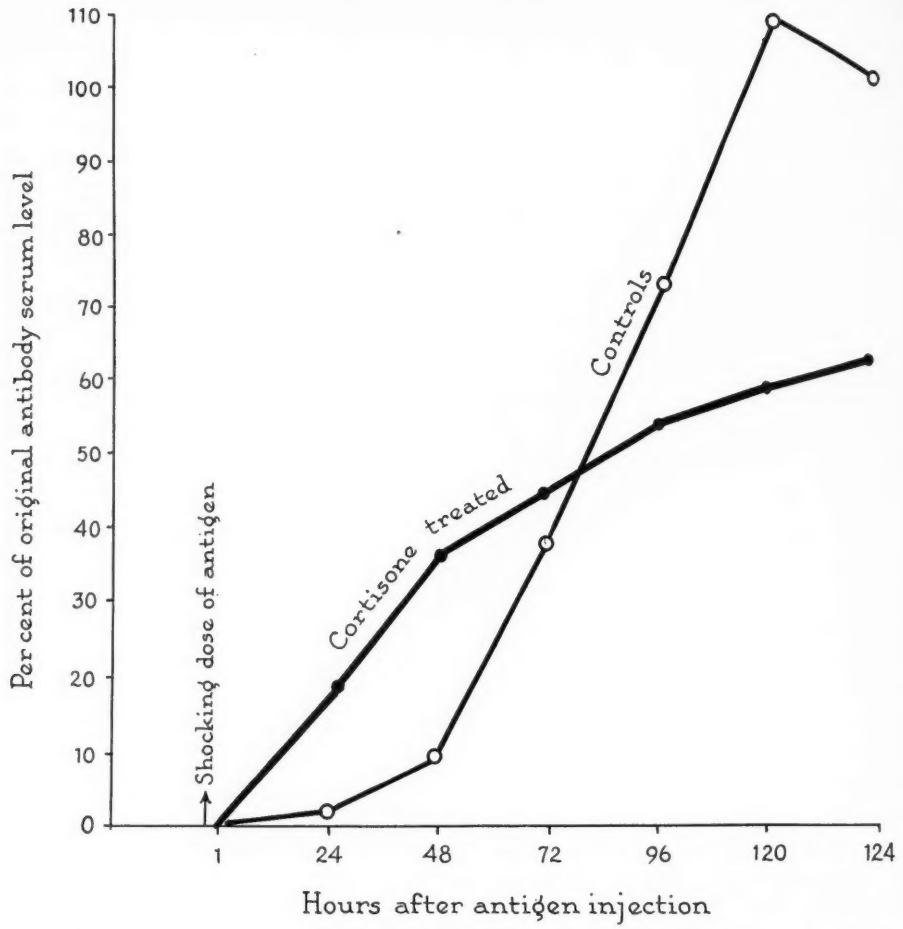

Fig. 1.—Modifications of serum antibody levels in sensitized rabbits treated with cortisone during the period following the intravenous injection of a saturating dose of antigen.

of the animals, i.e., loss of weight, cachexia, spontaneous infections, etc. *Could these alterations of the health of the animals explain the weakening of the antibody production?*

It seemed interesting to us to study the action of cortisone not upon the antibody production during the course of sensitization, but upon the antibody production of the sensitized animal when all the circulating antibody has been neutralized by an intravenous injection of an adequate dose of antigen. It is well known that under these conditions the antibody level of the serum returns rapidly after four or five days to a level similar to the one which existed before the neutralizing injection of the antigen. This method avoids prolonged treatment with the hormones. The results presented in this study* afford a new approach to the problem of the action of adrenocortical hormones on the antibodies in the blood.

*This study was conducted in the Department of Research of the University Medical Clinic; Chief of the Clinic, Prof. Pasteur Vallery-Radot.

TECHNIQUE

Twenty-four rabbits were divided into two lots of twelve each. The animals were injected intravenously three times a week for three weeks with doses of crystallized ovalbumin, increasing progressively from 0.5 mg. per kilogram to 2.0 mg. per kilogram. Eight days after the last injection the antibody levels of the sera were ascertained by the precipitin method. We determined the equivalent point of antigen-antibody for each serum, taking our readings after the tubes had remained forty-eight hours in the refrigerator. We expressed the antibody level of the sera by the amount of albumin necessary for a complete saturation of the antibodies. These values varied between 150 and 650 gammas per c.c. of crystallized ovalbumin. When the level of antibody had been established we injected intravenously a calculated amount of antigen to that which was thought necessary for a complete saturation of the antibodies. We then analyzed the sera for antibody levels one hour, 24, 48, 72, 96, 120, and 144 hours later.

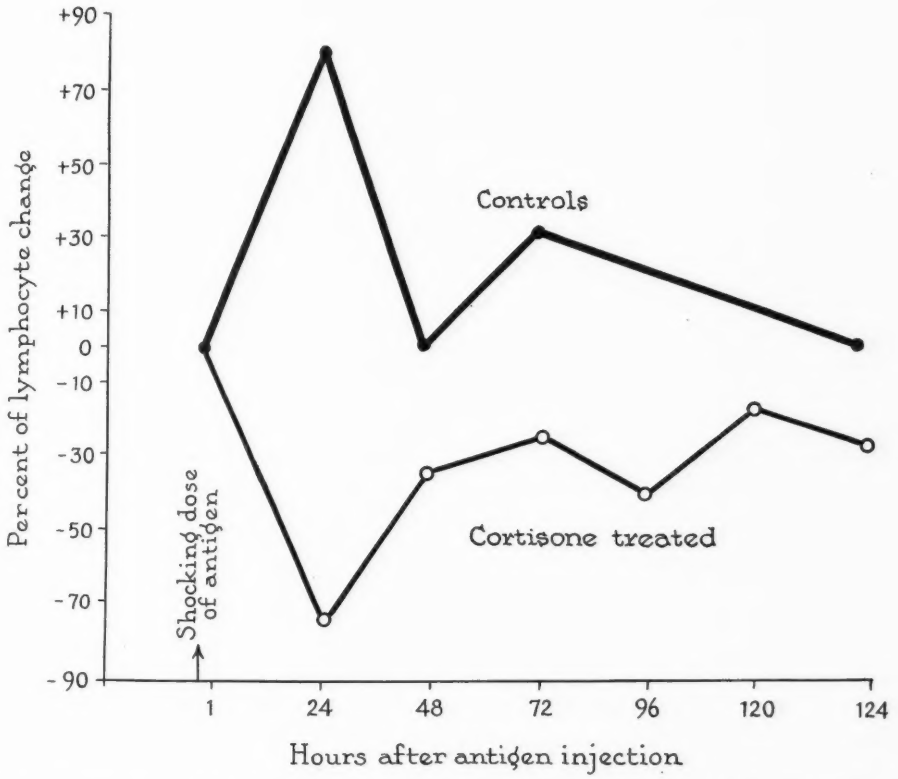

Fig. 2.—Modifications of levels of blood lymphocytes in sensitized rabbits treated with cortisone during the period following the intravenous injection of a saturating dose of antigen.

The cortisone-treated group received, 70 minutes after the injections of antigen and every day afterward, either 10 mg. or 25 mg. per kilogram of cortisone daily in two injections. The antibody levels were determined in these

animals in the same way as in the control group, and the histologic appearance of lymphatic tissues and the number of lymphocytes in the blood were studied as well in both groups of animals.

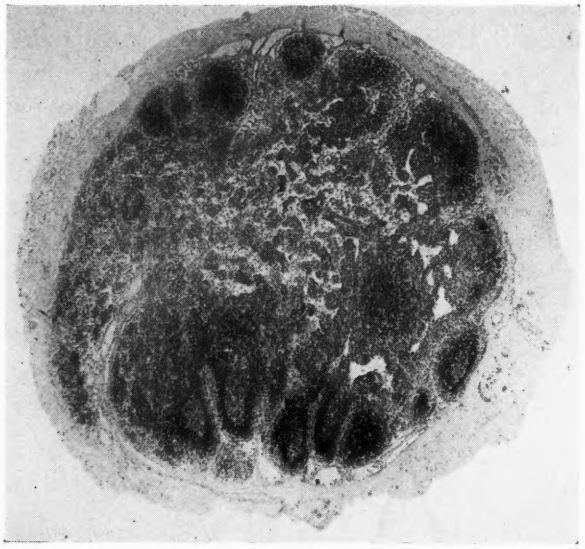

Fig. 3.—Normal lymph node: Under the capsule the lymph follicles with their paler germinative center and the crown of lymphocytes on the periphery. The lymphatic cords separated by the lymphatic sinuses converge toward the center of the node.

RESULTS

Changes in the Antibody Levels in the Control Animals After the Neutralizing Injection of Antigen.—As can be seen in Fig. 1, after the injection of a dose of antigen calculated to be theoretically saturating, the antibody level fell to the extent that there were no precipitins in the sera taken one hour after the injection. In the control animals, the antibody levels remained close to zero for 24 hours; after 48 hours, the level of precipitins came normally to about 8 per cent of the original amount, the level increased then rapidly to 79 per cent of the original amount after 4 days and after 6 days to more or less 100 per cent of the original amount.

Effect of Cortisone Upon the Increase of the Serum Antibody Level After the Neutralizing Injection of Antigen.—The results obtained were similar, whether the animals received 10 or 25 mg. of cortisone a day. As can be seen in Fig. 1, the results obtained with the rabbits treated with cortisone showed a great difference from those obtained with the control animals. After 24 hours the treated animals showed a serum level of 17.2 per cent of the original amount of precipitin, while the control animals showed a level of 0.9 per cent of the original amount of precipitin. After 48 hours, the treated animals had recovered on an average an antibody serum level of 36.6 per cent of the original amount, while the control animals had gained only 9 per cent. The third day, the two curves met and crossed each other at the 45 per cent level of recovery. But after the third day the antibody levels in the treated animals compared

to the control animals began to lag decisively and consistently. When the control animals had completely recovered their original level of antibody, generally after the fifth day, the treated animals showed only a level of precipitins of 56.4 per cent of the original amount.

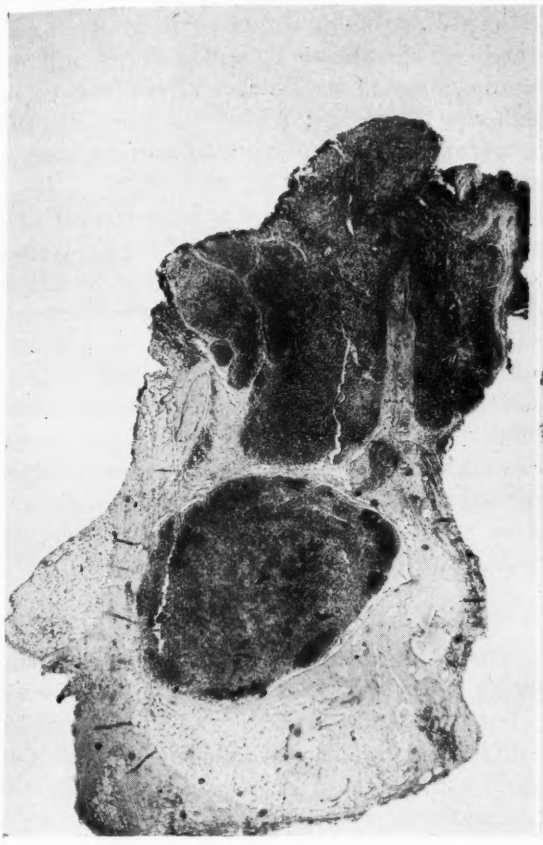

Fig. 4.

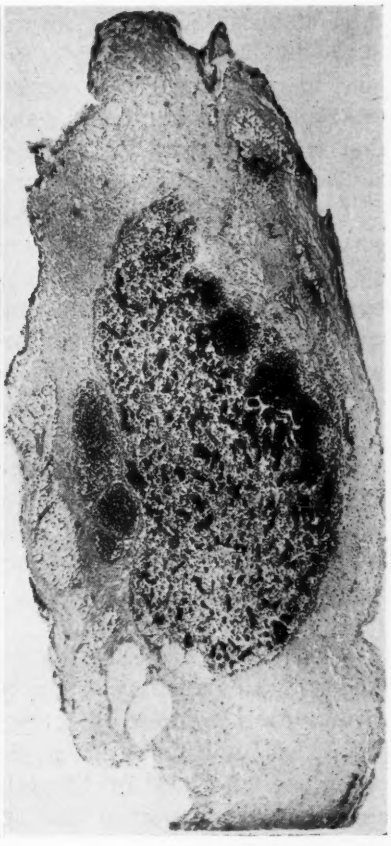

Fig. 5.

Fig. 4.—Lymph node of a rabbit one hour after the challenging dose of antigen. Decrease of the number of free cells associated with hyper-reticulosis. The lymph follicles and the lymphatic cords are not distinct.

Fig. 5.—Lymph node of a rabbit after six days of treatment with cortisone; considerable atrophy of the lymphatic elements. The lymph follicles and cords have almost completely disappeared. The lymphatic sinuses are empty and wide.

DISCUSSION

It is very clear that treatment with cortisone modifies definitely the pattern of recovery of the serum antibody level in the sensitized rabbit after the injection of a neutralizing dose of antigen. Two distinct modifications from the normal should be noted in the animals treated with cortisone. In the first 48 hours after the injection of the antigen, the antibody level is found to be consistently and significantly higher than in the control. But after the third day, there is a serious lag in the recovery of the serum antibody level in the treated animals compared with the controls.

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How could we explain this seemingly paradoxical behaviour of the animals treated with cortisone? We believe that the remarkable increase in the serum antibody level which is observed in the first 48 hours, is due to the release of antibodies under the stimulation of the hormones, from the lymphatic tissues. Indeed, we have been able to observe a considerable lysis of lymphocytes in these animals (Fig. 2). The parallel studies of the serum antibody levels and of the number of lymphocytes in the blood and tissues show that as the antibody level increases, there is a corresponding drop in the number of lymphocytes in the blood and lymph nodes. Dougherty, Chase, and White[5, 6] showed, in 1945, that treatment with ACTH and hormones from the adrenal cortex causes a sudden release of antibodies from the lymphocytes.

On the other hand, the modifications observed in the animals treated with cortisone, after the third day, i.e., the decrease after that date of the production of antibodies as compared with the controls, could be explained by an inhibition by the hormone of antibody production by the cells of the reticulo-endothelial system which produce them normally. This interpretation would be consistent with the data reported by other workers on the effect of cortisone upon antibody production during active sensitization. The results of our experiments afford a possible explanation for the conflicting findings of the authors who, on the one hand, have observed an increase of antibodies in the blood under the effect of cortisone during the anamnestic response, and on the other hand, those who have reported a depressing effect of this hormone on the production of antibodies when administered during the sensitization period.

SUMMARY

The effect of cortisone on the level of serum-antibodies in rabbits sensitized against ovalbumin during the period which follows the challenging injection of a neutralizing dose of antigen has been studied.

It has been found that in the first forty-eight hours following the injection of antigen the level of the antibodies in the sera of the treated animals is significantly higher than that of the control.

After the third day, on the contrary, the production of antibodies of the treated animals is found to be much lower than that of the control.

An explanation of these facts is suggested.

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MAINTENANCE CORTISONE IN SEVERE BRONCHIAL ASTHMA

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SEVERAL authors[1-6] have reported that cortisone and corticotropin will relieve attacks of asthma It is now common knowledge that asthma returns within varying periods of time once such therapy is terminated. The problem of maintenance therapy has more recently come under intensive investigation.[7, 8]

In our clinic there is a small group of people with constant, severe bronchial asthma in whom it has been impossible to uncover any specific antigenic factors and in whom routine antiasthmatic therapy has been so unsuccessful that repeated periods of hospitalization are required. It was determined to run a controlled clinical experiment with cortisone, keeping the following objectives in mind:

1. To bring about complete relief from asthma.
2. To prolong this freedom from asthma for a year or more by appropriate maintenance therapy.
3. To determine if undesirable pharmacologic effects of cortisone could be avoided.
4. To study whether cortisone produces any demonstrable effect upon the peripheral circulation.

METHODS

The available quantity of cortisone limited the study to six patients. In order not to influence the results of the experiment in any way, it was decided to offer treatment to the first six of this group who required hospitalization. Each received four days of the customary intensive emergency treatment. When no marked improvement followed, the possibility of cortisone therapy was discussed with each, including the danger and the nature of undesirable effects, as well as the possibility of failure. Furthermore, all had to promise the utmost in cooperation throughout treatment.

The following studies were undertaken on each patient: Chest x-ray and fluoroscopy, vital capacity, urinalysis, complete blood count, electrocardiogram, circulating eosinophil counts, fasting blood sugar, and the blood content of sodium, chloride, carbon dioxide, potassium, and nonprotein nitrogen. The blood vessels of the bulbar conjunctivae of each patient were repeatedly examined through a binocular microscope to observe the condition of the blood vessels, the cellular content of the blood, and the presence or absence of edema. Detailed histories and thorough physical examinations were recorded. All six were placed on a moderately low sodium diet with no added salt.

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Cortone Acetate used in this study was furnished through the courtesy of Merck and Co., Inc.

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As it is extremely difficult to judge the degree of improvement in asthma, it was determined that these patients must be completely free of asthma subjectively as well as upon physical examination. Since these six patients were to be treated as a group and given 24 hour medical service, it was realized that group therapy might play a role. Furthermore, maintenance treatment with cortisone in rheumatoid arthritis has been reported to lead to psychiatric difficulties.[9] It therefore seemed wise to follow these patients from a psychiatric viewpoint to evaluate any untoward emotional changes as well as the part group therapy might play.

Each patient was rendered free of asthma with cortisone. Studies were then repeated and the patient sent home on maintenance dosage. The patient was thoroughly examined each week. Once a month studies were repeated and doses lowered until asthma returned. Each patient was then maintained on an optimal dose.

CASE HISTORIES

CASE 1.—A. W.: A 56-year-old, white widow. In 1921, at the age of 25, she developed asthma during her second pregnancy. No specific antigenic factors were discovered at the Allergy Clinic of the Massachusetts General Hospital. She never completely cleared thereafter, even though she was able to carry on her duties as a housewife most of the time. She developed bronchopneumonia and severe asthma 19 years later. This required her first hospitalization of two weeks' duration at the Boston City Hospital. For the next year she was treated at an out-patient Allergy Clinic with vaccines, epinephrine, and potassium iodide. Her asthma continued to be persistent but mild. For the next nine years, the use of routine medications kept wheezing under control. Status asthmaticus developed on Nov. 23, 1950, at the age of 55, necessitating a second hospital entry, this time at the Massachusetts General Hospital. In spite of intensive therapy, no real improvement was noted in four days so she was given 20 mg. of corticotropin intramuscularly every eight hours. After improvement this dose was gradually reduced. On December 4, 1950, the hormone was discontinued and she was discharged almost free of asthma. Five weeks later she developed a cold, severe asthma returned, and the following week she was again admitted to the hospital in status asthmaticus. Partial clearing occurred on routine therapy and she was discharged in five days, only to be readmitted ten days later, again in status asthmaticus. Corticotropin was readministered in similar doses and a complete remission from asthma occurred within a week. On Feb. 12, 1951, she was discharged on a maintenance dose of 10 mg. corticotropin intramuscularly every 6 hours. Asthma returned, however, in spite of similar therapy at home, even though the dose was raised as high as 125 mg. daily. After ten weeks of unsuccessful maintenance treatment, she was again admitted to the hospital and withdrawn from corticotropin, whereupon her asthma grew more severe. Finally, in May of 1951 intramuscular cortisone therapy was started.

The past medical history was not contributory. The family history showed no asthma or allergic disease. Physical examination revealed a tall, well-nourished, white woman propped up in bed, struggling for breath and wheezing audibly. Her weight was 149, blood pressure 160/85, temperature 98.6°, respiration 28, and pulse 100. The face had a drawn, anxious expression; the A-P diameter of the chest was increased; the sternum was prominent and there was marked hollowing of the jugular notch and supraclavicular fossae; the lungs were hyper-resonant with inspiratory and expiratory musical râles and ronchi in all areas. The rest of the physical examination was within normal limits.

Studies Before Cortisone.—Vital capacity was 1 L. in 3 seconds; roentgenogram and fluoroscopy of the chest showed marked limitation of movement of the diaphragm with flattening; the lungs were bright with appearance of emphysema; roentgenogram of sinuses

revealed clouding of the right antrum; circulating eosinophils were 1226 per cubic millimeter; routine urinalysis and complete blood count were within normal limits; electrocardiogram was within normal limits; and multiple skin tests by scratch and intradermal methods were all negative.

TABLE I. BLOOD CHEMISTRY

	BEFORE CORTISONE	AFTER CLEARING OF ASTHMA	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.
Case A. W.											
Sodium	141	142	139	141	141	139	139	142	140	139	143
Potassium	5	4.3	4.4	5.4	4.4	4.4	5	3.9	4.6	4.7	4.6
Chloride	104	99	102	92	98	98	106	94	102	108	111
CO_2	29							28			
F.B.S.										83	
N.P.N.				25	23	23	27		26	27	26
Case P. B.											
Sodium	142	134	136	136	138	137	139	135	138	140	140
Potassium	4.4	4.8	5.1	4.2	4.1	4.4	4.3	6.2	4.5	3.9	4.6
Chloride	101	97	105	100	99	100	104	94	94	100	110
CO_2	29.8	32.9						28.8			
F.B.S.	96	94								98	
N.P.N.				35	30	25	43		24	25	29
Case M. D.											
Sodium	138.6	140		139.6	138.3	138	135	143	138	141	139
Potassium	4	4.3		4	4.6	5	4.3	4.1	4.4	3.9	4.6
Chloride	103	104		104	95	97	106	99	96	100	105
CO_2	27.8							29.6			
F.B.S.	94								75		
N.P.N.	22			30	38	21	22		25	18	26
Case N. Z.											
Sodium	141.6	142	142	139	141	140	134	139	139	139	140
Potassium	3.8	5	4.9	5.2	3.9	4.9	4.4	4.7	5.9	4.7	4.5
Chloride	106	99	100	97	100	96	97	90	102	108	100
CO_2	31.4	32								32	
F.B.S.	102									83	
N.P.N.	34	34	20	20	23	17	27		25	27	24
Case D. M.											
Sodium	139	140		140	139	142	140	139	139	139	138
Potassium	4.4	4		4.2	4.6	3.6	4.3	4.2	4.1	4.1	4.4
Chloride	103	104		107	102	104	98	98	95	95	101
CO_2	28							33			
F.B.S.	100									100	
N.P.N.	28	25		50	24	29	29		24	24	27
Case E. M.											
Sodium	139	139				133	140		141	139	141
Potassium	4.8	4.5				4	4		3.9	4.2	3.9
Chloride	99	99				102	105	96	104	102	108
CO_2											
F.B.S.	73									75	
N.P.N.	29	25				25	30		20	38	27

Sodium, potassium,, chloride, carbon dioxide, and blood glucose all expressed in meq/liter. N.P.N. expressed in mg. per cent.

Normal values for these methods in this laboratory:

Sodium	136-145 meq/liter
Potassium	3.0-5.5 meq/liter
Chloride	100-106 meq/liter
Carbon dioxide	26-28 meq/liter
Fasting Blood Glucose	70-100 mg. per cent
N.P.N.	15-35 mg. per cent

Course on Cortisone.—On May 5, 1951, intramuscular cortisone was started. Table II shows that the patient cleared completely on the eighth day of therapy after a total dose of 1.15 Gm. Fig. 1 illustrates her dosage schedule through the months. On Dec. 1, 1951, she was withdrawn from cortisone as asthma had not returned after weeks of small single

daily doses. She remained symptom-free without any medication for two months until a head cold developed Jan. 30, 1952. Asthma returned two days later whereupon she was again placed on cortisone. Within 48 hours she was clear once again.

CASE 2.—P. B.: A 69-year-old, white married man. His first attack of asthma appeared at the age of 59 in May of 1941. At first only one or two attacks occurred daily, one of which usually appeared immediately after supper. These were promptly relieved with epinephrine. The following year his asthma became more persistent. He frequently had to sit up at night for several hours in spite of ephedrine, epinephrine, and hydriodic acid. He struggled on in this manner until 1949 when, at the age of 67, his asthma took a turn for the worse. He became a frequent resident at his local hospital, lost weight, could not eat, and finally was forced to give up work. During one attack he lost consciousness and was rushed to the hospital where he was on the danger list for several days. On partial recovery, he flew to Tucson, Arizona, where he remained for eight months. There he improved on daily aminophylline, but his asthma remained persistent. On returning home, he managed to exist on epinephrine every 3 hours and intravenous aminophylline twice daily. Nasal polyps were removed on one occasion, but without affecting his asthma which continued to be intractible. He was admitted to the Massachusetts General Hospital on April 30, 1951. The patient's medical history was not contributory, but the family history showed that both his son and wife had asthma. Physical examination showed a well-nourished man looking older than his stated age, sitting up in bed and wheezing audibly. His weight was 153; blood pressure 160/90; temperature 98.6°, respiration 24, and pulse 60. The nose showed polypoid tissue; A-P diameter of the chest was increased; excursion of the diaphragm was limited; the lungs were hyper-resonant with inspiratory and expiratory wheezes; and the pulse showed bradycardia. The rest of the physical examination was within normal limits.

Studies Before Cortisone.—The venous pressure was 110 mm. of saline, and the vital capacity, 2.2 L. in 3 seconds. Roentgenograms of the teeth showed slight periodontoclasia; sinuses, slight thickening of the mucous membrane of both antra; chest, clear and emphysematous. The heart was slightly round (both left and right ventricle), and the aorta was elongated and tortuous. An electrocardiogram revealed bradycardia; circulating eosinophils, 360 per cubic millimeter; routine urinalysis and complete blood count were normal; and multiple skin tests by the scratch and intradermal methods were all negative.

Course on Cortisone.—On May 3, 1951, he was started on intramuscular cortisone. Table II points out that clearing occurred in 6 days on a total dose of 0.825 Gm. Fig. 1 shows his maintenance schedule through the months. It appears that he did best on a total daily intramuscular dose of 62.5 mg. divided into three doses. We have not been able to withdraw him from cortisone. On the correct maintenance dose, however, he has been completely free of asthma.

CASE 3.—M.D.: A 56-year-old white widow. She developed asthma in 1926 at the age of 29, two months before the birth of her third child. For the first two months the wheezing was mild and easily controlled, but it then became more severe and, during the following four years, recurred in monthly attacks. Three of these required hospital admissions. Wheezing persisted for the next nineteen years, but she managed to do her work as a housewife until December, 1949, when at the age of 53, her asthma became constant and severe. During the next eighteen months she was admitted to the Massachusetts General Hospital eleven times. On various occasions she received courses of intramuscular or intravenous corticotropin with improvement each time, but her asthma always became severe within a few days after withdrawal of the hormone. On May 18, 1951, she had her twelfth admission, and, upon failure to improve with the usual therapy, was placed on intramuscular cortisone.

The medical history was not contributory, but the family history showed that a sister had urticaria, and a brother had hay fever.

Physical examination showed a poorly-nourished woman who looked older than her stated age of 54, gasping for breath and wheezing. Her weight was 85; blood pressure 170/90; pulse 116; respiration 30.

TABLE II. INITIAL COURSE OF CORTISONE TO FREE PATIENTS OF ASTHMA

PATIENT	AGE	SEX	ROUTE	1ST	2ND	3RD	4TH	5TH	6TH	7TH	8TH	9TH	10TH	11TH	12TH	13TH	14TH	TOTAL GM.
A. W.	55	F	I. M.	150	250	200	200	125	75	75	75							1.150
P. B.	69	M	I. M.	150	250	175	125	100	100									0.825
M. D.	55	F	I. M.	225	225	100	100	75										0.725
N. Z.	51	F	Oral	100	200	300	200	250	300	300	225	200	175	75	75	75	75	2.525
D. M.	57	M	Oral	150	250	200	200	150										0.950
E. M.	36	F	Oral	150	250	150	75											0.625

All daily doses divided into 4 (every 6 hours). Figures in mg.
First day dosage small as therapy started at noon.

The chest showed the A-P diameter to be increased and the diaphragmatic excursion limited; the lungs were hyper-resonant, and full of inspiratory and expiratory wheezes and rhonchi.

The rest of the physical examination was within normal limits.

Studies Before Cortisone.—The venous pressure was 48 mm. saline, and the vital capacity 1.8 in 3 seconds.

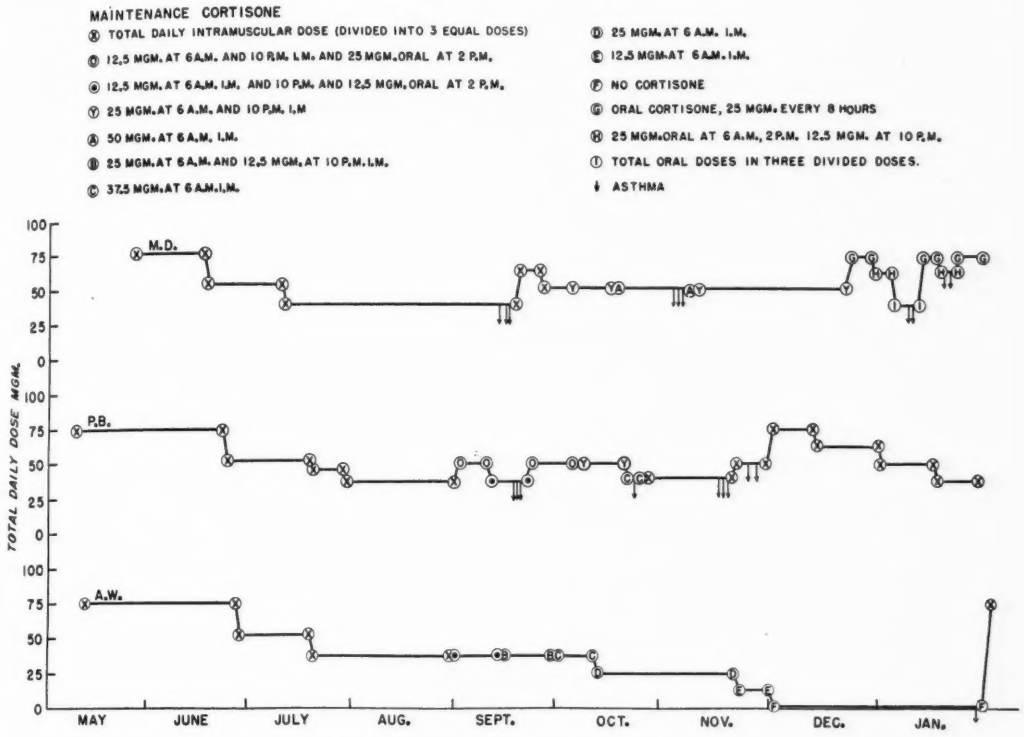
MAINTENANCE CORTISONE
TOTAL DAILY INTRAMUSCULAR DOSE (DIVIDED INTO 3 EQUAL DOSES)
12.5 MGM. AT 6 A.M. AND 10 P.M. I.M. AND 25 MGM. ORAL AT 2 P.M.
12.5 MGM. AT 6 A.M. I.M. AND 10 P.M. AND 12.5 MGM. ORAL AT 2 P.M.
25 MGM. AT 6 A.M. AND 10 P.M. I.M
50 MGM. AT 6 A.M. I.M.
25 MGM. AT 6 A.M. AND 12.5 MGM. AT 10 P.M. I.M.
37.5 MGM. AT 6 A.M. I.M.
25 MGM. AT 6 A.M. I.M.
12.5 MGM. AT 6 A.M. I.M.
NO CORTISONE
ORAL CORTISONE, 25 MGM. EVERY 8 HOURS
25 MGM. ORAL AT 6 A.M., 2 P.M. 12.5 MGM. AT 10 P.M.
TOTAL ORAL DOSES IN THREE DIVIDED DOSES.
ASTHMA
TOTAL DAILY DOSE MGM.
M.D.
P.B.
A.W.
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.

Fig. 1.—Maintenance cortisone in severe asthma.

Roentgenogram and fluoroscopy of the chest revealed bright, full lungs as well as limited motion of the diaphragm. Circulating eosinophiles were 434 per cubic millimeter; routine urinalysis, complete blood count, and electrocardiogram were within normal limits. Multiple skin tests by the scratch and intradermal methods were all negative.

Course on Cortisone.—On May 22, 1951, she was placed on intramuscular cortisone. Table II shows that she cleared on 0.725 Gm. in 5 days. Fig. 1 charts her maintenance dosage throughout the months. Her maintenance dose proved to be 25 mg. intramuscularly twice daily although, surprisingly enough, 50 mg. given once daily did not maintain her free of symptoms. On Dec. 14, 1951, she developed an abscess of her right thigh at the site of an injection of cortisone, and on Dec. 24, 1951, a similar abscess appeared upon her left thigh. She was therefore switched to oral cortisone on Dec. 27, 1951. Interestingly, she required a total dose of 75 mg. of oral cortisone to remain free of asthma.

CASE 4.—N. Z.: A 51-year-old divorced, white woman. In 1936, at the age of 36, she developed bronchopneumonia followed by asthma. For the next ten years, mild wheezing occurred only on first getting up in the morning, but then she began to wheeze throughout the day.

Vasomotor rhinitis was added to the picture in 1947. Nasal polyps were found and removed at that time. Two years later, at the age of 49, asthma became severe and constant, and during the next three years she was admitted to hospitals on three occasions. In January and February of 1951, short courses of cortisone were helpful, but on withdrawal of the hormone, symptoms returned in a few days. In late February, 1951, she had four days of intravenous corticotropin with temporary relief. On April 21, 1951, she was admitted to the Massachusetts General Hospital. Routine therapy was not helpful so on April 25, 1951, she was put on oral cortisone.

The medical history showed pleurisy at the age of 16, deafness since the age of 20, and urticaria since the age of 50. The family history showed that one daughter had severe asthma. Examination showed a ruddy faced white woman in her early fifties sitting up in bed wheezing. Her weight was 125; blood pressure 130/90; respiration 35; pulse 95.

The nose showed polypoid tissue on both sides; the ears showed bilateral deafness; the chest showed increased A-P diameter, and limited diaphragmatic excursion.

The expiratory phase was prolonged; the lungs were hyper-resonant, with inspiratory and expiratory wheezes and rhonchi throughout. The rest of the physical examination was within normal limits.

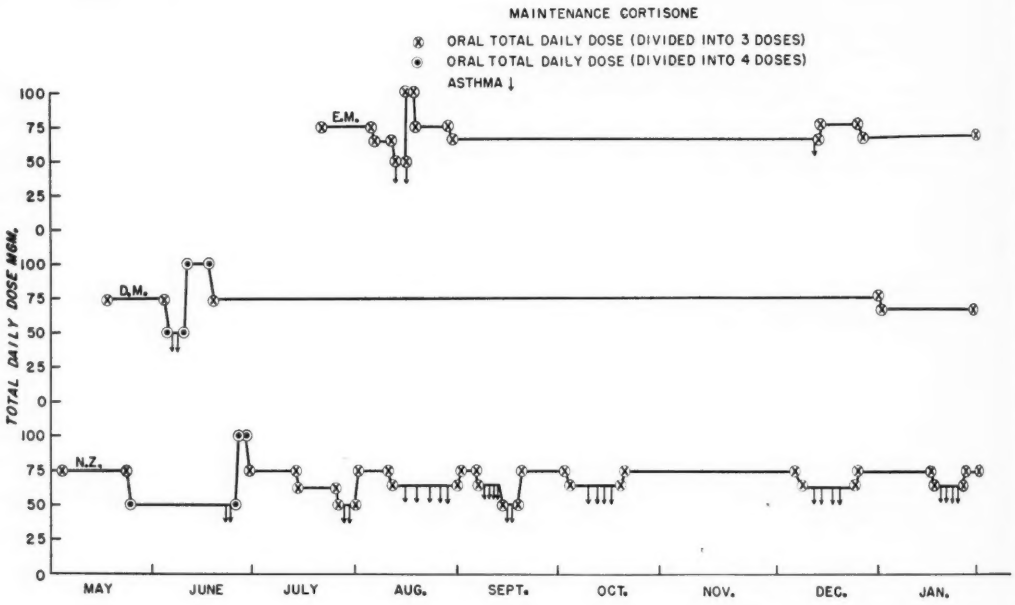

Fig. 2.—Maintenance cortisone in severe asthma.

Studies Before Cortisone.—The venous pressure was 130 mm. water and the vital capacity, 1.9 L. in 3 seconds. Roentgenogram of chest showed clear lung fields except for tiny calcification in right upper lobe, tortuous aorta, sclerosis and expansion T-10.

Circulating eosinophils were 1816 per cubic millimeter; routine urinalysis was within normal limits; complete blood count was normal except for 18 eosinophils; electrocardiogram was normal. Multiple skin tests by the scratch and intradermal methods were all negative.

Course on Cortisone.—Oral cortisone was started April 25, 1951. Table II shows that she cleared after 14 days with a total dose of 2.525 Gm. Fig. 2 shows that she needed 25 mg. every 8 hours to remain asthma-free.

CASE 5.—D. M.: A 57-year-old white, married man. Asthma first appeared in 1949 when, at the age of 55, he noticed wheezing on first getting up in the morning. This grad-

ually increased in frequency and intensity until the following year when it became severe and persistent. Epinephrine and ephedrine helped temporarily, but his course was steadily downhill. On Oct. 31, 1950, he was admitted to the Massachusetts General Hospital where a course of intramuscular corticotropin cleared his asthma, but within a few weeks he was in trouble again. He was readmitted on Nov. 9, 1951. Failing to improve on routine therapy, he was placed on oral cortisone.

The past medical history was not contributory and there was no family history of allergy or asthma. Examination showed a stout man in his late fifties sitting up in bed wheezing. His weight was 168; blood pressure, 130/90; temperature, 98.6°; pulse, 90; and respiration, 25. The chest showed an increased A-P diameter, and the diaphragmatic excursion was limited. The lungs were hyper-resonant, with inspiratory and expiratory wheezes and rhonchi throughout. The rest of the physical examination was within normal limits.

Studies Before Cortisone.—The vital capacity was 2.1 L. in 3 seconds. Roentgenogram and fluoroscopy of chest revealed bright, large lungs and flattened diaphragm. Circulating eosinophils were 281 per cubic millimeter; routine urinalysis and complete blood count were normal, except for 16.5 grams hemoglobin of blood.

The electrocardiogram was within normal limits, and multiple skin tests by the scratch and intradermal methods were all negative.

Course on Cortisone.—On May 10, 1951, he was placed on oral cortisone. As shown in Table II, he cleared in five days after 0.950 Gm. Fig. 2 shows that his maintenance dose was 25 mg. every 8 hours.

CASE 6.—E. M.: A 36-year-old white, married woman. Her asthma appeared for the first time in 1941 at the age of 26, during her second pregnancy. This attack only lasted for 24 hours after which she cleared. There was no further difficulty until three months after the birth of the child when asthma returned. She was admitted to the Peter Bent Brigham Hospital after eight weeks of severe, persistent asthma. Three weeks of hospital care was required at that time. From then on chronic asthma persisted. In early 1951 she was hospitalized for three weeks at the Massachusetts General Hospital. Asthma persisted and again in July she was admitted. During this hospital stay she was given a small dose of aspirin and nearly died from severe asthma. Immediate intravenous corticotropin was necessary to control this attack. Following its withdrawal, her asthma soon returned, and she was placed on oral cortisone.

There was a past history of eczema as a child, and pneumonia at the age of 13. Her father died of asthma, and her father's sister had asthma. Examination showed a white woman in her thirties sitting up in bed wheezing. Her weight was 127; blood pressure, 86/60; pulse, 90; respiration, 24; temperature, 98.6°. The A-P diameter of the chest was increased, and the lungs showed inspiratory and expiratory wheezes and rhonchi in all fields. The rest of the examination was not contributory.

Studies Before Cortisone.—The vital capacity was 2.1 in 3 seconds. Roentgenogram of chest showed large and bright lung fields and a low diaphragm. The circulating eosinophils were 127 per cubic millimeter and the electrocardiogram was within normal limits.

Course on Cortisone.—On July 17, 1951, she was placed on oral cortisone. She cleared after 0.625 Gm. was given in four days as shown in Table II. Her maintenance level seemed to be 62.5 mg. per day.

PHARMACOLOGIC EFFECTS

Sprague and associates[10] preferred to speak of the physiologic effects of cortisone and corticotropin rather than therapeutic and side effects. We choose, however, to group all actions of cortisone under the title of pharmacologic effects as this hormone is probably used in disease in amounts larger than those normally found in the body.

All six patients have been relieved of asthma completely, and have been maintained free of asthma for months on daily doses of cortisone.

Table III shows that all six have gained weight. In no instance has there been evidence of gross edema. These patients had been ill for years. Then, when they were well, food suddenly was attractive. Every effort was made to keep their calorie intake at a reasonable level, but people are human. It is of interest to note that only D. M. and M. D. have so far exceeded their previous maximum life weight.

TABLE III. WEIGHT IN POUNDS

	MAXIMUM WEIGHT IN LIFE BEFORE CORTISONE	WEIGHT JUST BEFORE CORTISONE THERAPY	WEIGHT JANUARY, 1952, AFTER MONTHS OF CORTISONE
A. W.	164	147	150
P. B.	160	150	170
M. D.	130	85	139
N. Z.	160	125	143
D. M.	170	168	179
E. M.	175	127	162

All six patients have developed minimal rounding of facial contours. Furthermore, N. Z. has grown a small, light amount of facial hair. None have shown acne or cutaneous striae. Muscular weakness has not appeared. Of the two patients still menstruating, N. Z.'s periods were irregular before cortisone, but are now quite regular; E. M.'s menstruation formerly lasted five days and is now but three; P. B. is the only one to note an increase in libido—the others have observed no change. There has been no alteration in blood pressure in any of the six. In December, 1951, a glucose tolerance test was performed on each patient, and in each instance a normal curve was obtained.

Table I shows the blood chemistry done throughout the study. No significant changes were noted. We realize that measuring the potassium of the plasma does not give direct evidence as to the potassium concentration within the cells.

In connection with the effect of cortisone on circulating eosinophils, certain observations are of interest:

1. A. W.—Before her first dose of cortisone, the circulating eosinophil count was 521 per cubic millimeter. Forty-eight hours later the count was 1396 per cubic millimeter, just two hours after a dose of cortisone. Recent counts still show elevated readings.

2. P. B.—The count was 360 per cubic millimeter before cortisone therapy. One hour after a dose of cortisone is was 2 per cubic millimeter.

3. M. D.—Prior to cortisone, her count was 434 per cubic millimeter. It now runs in the vicinity of 300 to 400 per cubic millimeter.

4. N. Z.—Before cortisone her count was 1816 per cubic millimeter. After considerable cortisone and with asthma still present, the count dropped to 11 per cubic millimeter. It now is elevated again.

5. D. B. showed a count of 281 per cubic millimeter before cortisone with a fall to 31 after cortisone had controlled his asthma.

6. E. M.'s original reading was 127 per cubic millimeter. This rose to 510 after cortisone had cleared her asthma.

P. B. and N. Z. had nasal polyps which cleared on cortisone. Roentgenograms and fluoroscopy of the chest of all six in December of 1951 suggest that all have better excursions and higher positions of their diaphragms than before cortisone. The brightness of the lung fields showed no change in A. W., M. D., D. M., and E. M. after cortisone, whereas the lungs of P. B. and N. Z. were brighter than before treatment. Vital capacities in three seconds were as follows:

	BEFORE CORTISONE	AFTER CORTISONE
A. W.	1	2.2
P. B.	2.2	2.8
M. D.	1.8	2.6
N. Z.	1.9	2.8
D. M.	2.1	3.8
E. M.	2.1	3.2

While these studies are not considered adequate evidence of increased pulmonary function per se, they do tend to confirm clinical observations that these patients have all materially increased their exercise tolerance.

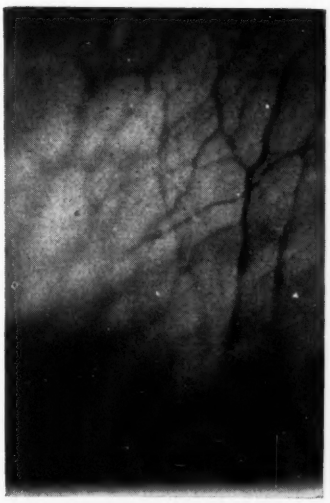

Fig. 3.—Some of these clumps at 60 times magnification in a small vein of the bulbar conjunctiva of one of the patients on cortisone.

Knisely and Bloch[11] have studied the peripheral circulation of the bulbar conjunctiva of human beings using reflected light and a binocular, stereoscopic microscope. With similar techniques, we observed the vessels and circulating blood of the bulbar conjunctivae of these six patients before cortisone and at weekly intervals during therapy. Shortly after the onset of treatment, clumping of the cellular elements of the blood was noted. This clumping has never cleared during therapy. At times the clumps have been seen to plug the ends of small arterioles intermittently. We have also noted edema of the tissues about the vessels during cortisone therapy.

Each patient has been studied from a psychiatric standpoint by one of us (J. G.). His observations indicate that A. W. has shown slight euphoria on cortisone. In a like manner, P. B. has exhibited a definite euphoria, tending to activity a little in excess of his physical capacity, while M. D. showed a similar tendency expressed in her ability to socialize. Neither N. Z. nor D. M. gave any suggestion of euphoria at all, although E. M. showed signs of it on the basis of once again being in high spirits. None of these patients has given any evidence of basic mental changes while on maintenance cortisone therapy. He felt that group therapy played a part in our success with cortisone therapy.

COMPLICATIONS

All six have had upper respiratory infections. E. M. developed a head cold in December which progressed to a tracheobronchitis. She was therefore admitted to the hospital for observation. H. influenza was isolated from her sputum, a chest plate was noncontributory, and she recovered uneventfully with no change in her cortisone dosage. P. B. developed a cheilosis in October of 1951 which responded to vitamin therapy. M. D. developed two abscesses, both at the sites of previous injections of cortisone. These had to be incised and drained and necessitated placing her on oral cortisone as described earlier.

DISCUSSION

Cortisone has completely relieved the severe longstanding bronchial asthma of these six patients and suitable daily maintenance doses have enabled them to remain symptom free for many months. All of these cases have been ones in whom no specific etiological diagnosis could be made nor have we or others been able to treat them successfully with the routine methods. Figs. 1 and 2 clearly show that each patient has an optimum maintenance level. Five of the patients have needed cortisone at least once a day. In most instances, it seemed best to divide their daily doses into three equal parts. In the cases of M. D. and P. B., the table suggests that intramuscular cortisone is more effective milligram for milligram, than is the oral route. One patient remained symptom free for an eight week period after withdrawal of cortisone only to have a recurrence of asthma with an acute respiratory infection.

These patients have been returned to a useful place in society. All are now at work in paying positions. Undesirable pharmacologic effects of the hormone have not presented themselves in any marked degree up to the present time.

Knisely[12] has described clumping of red blood cells which he calls sludge. Clumps have appeared in the circulation of these patients after they were placed on cortisone. We are not as yet prepared to say what these clumps may be nor have we found evidence to suggest that they are harmful.

SUMMARY

1. Six patients with severe bronchial asthma have been cleared with cortisone.

2. It has been possible to maintain these patients free of asthma for months with suitable daily doses of cortisone.

3. No untoward pharmacologic effects of cortisone have been noted to date.

4. Cortisone does seem to precipitate clumping of the cellular elements of the blood of the bulbar conjunctiva and to cause edema to appear in these areas.

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SUDDEN DEATH FROM BRONCHIAL ASTHMA FOLLOWING INJECTION OF PYROMEN

Case Report*

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THERE have been many reports of death from asthma.[1] This case is reported because of its somewhat unusual circumstances.

The patient was a white married woman, thirty-one years old at the time of her death (Sept. 14, 1951). She had been under nearly continuous observation since April 16, 1948. During her first pregnancy in September, 1945, she suffered symptoms suggestive of asthma but no record existed of such a diagnosis. At that time she was said also to have toxemia with hypertension. She remained well after that until early in her second pregnancy when in July, 1947, she developed allergic rhinitis with early polyp formation. In Sept., 1947, she had mild asthma and this recurred at intervals until her second baby was born in February, 1948. In March, 1948, she underwent a nasal polypectomy and a bilateral radical antrostomy. Following this, her asthma became very severe and intractable. There was no known family history of allergy.

The patient was depressed and anxious and there was a considerable amount of domestic discord. Her physical examination showed the usual pulmonary findings of severe asthma. The nasal mucosa was reddened and there was evidence of continued polyposis. A mucopurulent nasal discharge was noted. There was moderate arterial hypertension and her ocular fundi showed arteriolar changes, classified as grade I. She was underweight. Blood examination revealed a hemoglobin content of 11.8 Gm., 4,010,000 red blood cells, 8,350 leukocytes, and of these 16 per cent were eosinophils. Urinalysis yielded normal findings.

She was tested to the common inhalants, intradermally, but failed to react except very slightly to some fungus spores, feathers, kapok, and tobacco. The history indicated marked aspirin sensitivity. A test elimination diet was carried out. The results of this were inconclusive but the patient was certain that she did not tolerate wheat, onion, chocolate, and egg and usually left these articles out of her diet.

During the early phase of her management she was deeply disturbed psychologically. She suffered from anxiety reactions, personality conflicts, feelings of inferiority, tension, insecurity, and an inability to get along with her husband. In June, 1948, she took an overdose of bromides, accidentally, and this led to an acute psychosis necessitating her admission to the psychopathic hospital. Up to this time her asthma had been severe and nearly intractable. When she became psychotic her asthma subsided almost completely and all abnormal physical signs disappeared from her chest. She recovered from her acute psychotic episode quickly and was discharged within seventeen days. Immediately her asthma returned so severely that she required several hypodermic injections of epinephrine daily.

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For the next six months she presented a difficult therapeutic problem but was able to carry on at home. She had a remission in March and April, 1949 but during the following summer had gradually increasing asthma. During this time and the following winter she required periodic relief of intercurrent infections with antibiotics and with frequent use of symptomatic measures carried on fairly well until a very severe asthmatic state necessitated admission (her first in thirty months) to hospital in November, 1950.

Fig. 1.—Section through lung root showing mucosal thickening and mucous plugging in secondary bronchi, vesicular emphysema and enlarged bronchopulmonary nodes.

There was moderate improvement while in the hospital but she relapsed soon after discharge and readmission was necessary on February 10, 1951. Her inability to respond to any medication led to the use of ACTH. After 500 mg. given over a period of five days, she became free of all signs and symptoms. Her psychological problems went into abeyance and she seemed well in every way. Within two weeks need of medication returned. However, she managed to carry on with only minimal difficulty until late March, 1951, when symptoms again increased considerably and were almost intractable on her admission to hospital again on July 6, 1951. She received 280 mg. ACTH over a period of four days, most of it intravenously, and made another dramatic recovery, going home free of signs and symptoms on July 12, 1951. Symptoms began to recur on July 31, 1951, and increased steadily. These symptoms were aggravated by much vomiting and she was readmitted to hospital on August 31, 1951, because of vomiting and a fainting attack.

Her asthma was not especially severe. Epinephrine, rectal aminophyllin and inhaled Isuprel was required daily but she was able to be up and about the ward and she was not as severely incapacitated as on previous occasions. Signs of asthma were constantly present in her chest, but dyspnea was moderate and she slept well. Her condition did not warrant the use of ACTH.

TABLE I.

DATE	INTRAVENOUS PYROMEN	REACTION
7 Sept. 51.	1 microgram (0.1 c.c.)	Chilliness. T.99°F.
8 Sept. 51.	2 micrograms (0.2 c.c.)	Slight shivering. T.99.3°F.
9 Sept. 51.	3 micrograms (0.3 c.c.)	Slight shivering. T.99.3°F.
10 Sept. 51.	3 micrograms (0.3 c.c.)	No reaction
11 Sept. 51.	5 micrograms (0.5 c.c.)	Flushed—no elevation of temperature.
12 Sept. 51.	3 micrograms (0.3 c.c.)	No reaction
13 Sept 51.	None used	
14 Sept. 51.	5 micrograms (0.5 c.c.)	No immediate reaction—then rapidly increasing dyspnea and death in 85 minutes.

It was decided to give her a trial on Pyromen* therapy,[2] and this was done as shown in Table I. No immediate reaction occurred following her last intravenous injection of Pyromen and although she still manifested asthmatic

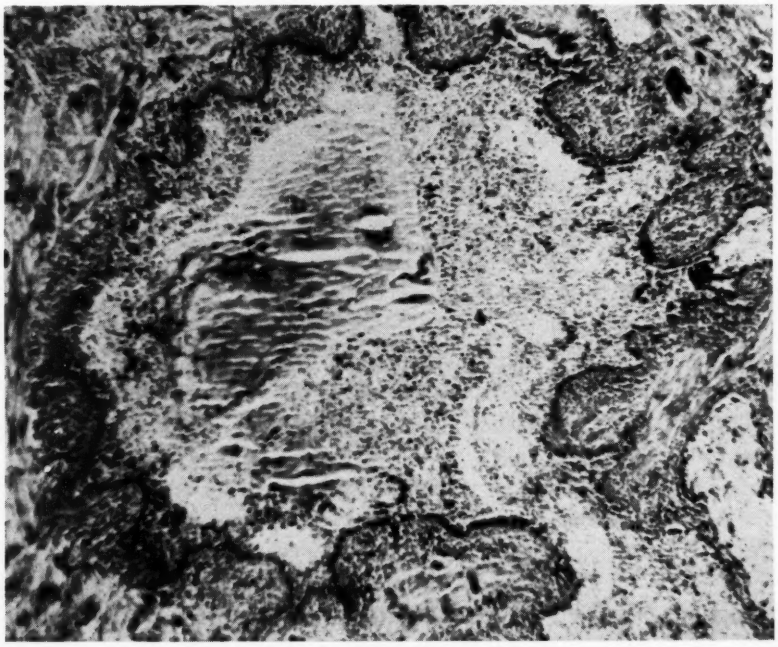

Fig. 2.—Tertiary bronchus, lumen 2 mm., occluded by mucus which contains numerous eosinophils. Dark wavy line made up of nuclei of epithelium, the free border of which is ruptured. At the lower right corner is a distended mucous gland duct.

signs throughout her chest she was not specially dyspneic nor was she cyanosed. Shortly afterwards, dyspnea increased and she asked for epinephrine. Up to this time the ward staff were not alarmed, but when epinephrine in repeated

*Pyromen is a nonprotein bacterial pyrogen, the active factor in which appears to be a complex polysaccharide. It is prepared by the Baxter Laboratories, Inc., Morton Grove, Ill. and distributed by Travenol, Inc. The material used in this case was provided by Travenol, Inc.

doses was without effect, administration of intravenous aminophyllin was requested because of rapid deterioration in her condition. Before this could be started she suddenly became deeply cyanosed, breath sounds became almost inaudible and she died suddenly one hour and twenty-five minutes after the Pyromen had been injected intravenously.

Fig. 3.—Section of wall of tertiary bronchus. From bottom to top: circular muscle and edema; lamina propria, eosinophil and lymphocyte infiltration; basement membrane markedly thickened; bronchial epithelium with cytoplasmic rupture. Lumen filled with mucus containing eosinophils, lymphocytes and shed epithelial cells. Magnification ×206.

AUTOPSY

An autopsy (W. G. H. A-8363) was performed to exclude common causes of sudden death, confirm the diagnosis of bronchial asthma, and to observe any effect of Pyromen.

External examination was normal except for cyanosis of the face and nail beds, evident twenty-four hours after death. The cardiovascular system showed heart weight of 240 grams, right ventricle wall 2 mm. thick, no dilatation of either ventricle, no valvular, coronary artery, myocardial, arterial or venous abnormality. There was no thrombosis or embolism; pulmonary vessels and femoral veins contained fluid blood. The kidneys showed congested parenchyma but were otherwise normal as were the ureters, bladder, and pelvic organs.

The gastrointestinal tract, liver, pancreas, spleen, and adrenals were normal. As the patient had been in hospital for a period of two weeks no examinations for poisons were carried out.

Skull, meninges, and brain showed no lesion except for a few petechial hemorrhages over the right posterior parietal region. Microscopic examinations of all brain sections and the pituitary showed marked congestion. The thyroid weighed 15 grams and contained colloid. The thymus weighed 20 grams, was bilobed with a soft pink cut surface.

The nares and fauces were patent, and the tongue not swollen or bitten. The larynx, trachea, and main bronchi were congested and contained a little clear sticky mucus but were not obstructed. The whole lungs were voluminous and crepitant. After fixation by formalin perfusion and immersion they were sliced at 4 mm. intervals, and showed findings characteristic of bronchial asthma, namely, most secondary and tertiary bronchi completely occluded by thick tenaceous plugs, and a fine honeycomb appearance of the parenchyma characteristic of emphysema. No atelectasis, consolidation, or scars were seen. The pleura was normal. Bronchopulmonary lymph nodes were enlarged to 2 by 1.5 by 1.5 cm., in contrast to the other nodes of the body which were average.

Microscopic examination showed diffuse mucus plugging of medium and small bronchioles with rupture of lining epithelium, swollen basement membrane, prominent mucus glands with ducts greatly distended by mucus, marked bronchial wall edema and alveoli distended, and empty except for a few free mononuclear cells. Numerous eosinophils were noted in hilar lymph node sinuses, lung venules, bronchial walls, and lumen where they were most numerous near the mucosa.

Except for visceral congestion the only pathological findings of importance were those of the lungs which presented the picture characteristically seen in sudden death from asthma.[1]

SUMMARY

1. A case of sudden and unexpected death from asthma following the intravenous use of Pyromen is presented. This young woman had an intrinsic type of asthma starting in pregnancy and becoming very severe after a double radical antrum operation. She was aspirin sensitive.

2. The patient had been psychologically unstable and during one acute psychotic phase became temporarily and completely free of asthma.

3. Two courses of ACTH, in February and July, 1951, respectively, produced complete remissions of asthma for a few weeks and led to temporary psychological stability.

4. Her fourth admission to hospital was characterized by less severe asthma than on previous occasions. Therapy with Pyromen produced no beneficial effects of any kind and her last treatment with this substance was followed by very rapidly increasing bronchial obstruction and death within eighty-five minutes. The fatal termination was not anticipated because although she had required daily medication her asthma was not severe at the time and she was ambulant.

5. Asthmatic patients of this type have a notoriously bad prognosis and often require very little to upset their delicately balanced respiratory state. Whether or not the use of Pyromen was causally related to her death it is impossible to say. It can be said with certainty that Pyromen had no demonstrable beneficial effect.

6. The pathological findings were limited to the respiratory tract. These showed a striking example of intense bronchial and bronchiolar obstruction due to mucus plugging and mucosal edema. The thymus was persistent. Death was due to asphyxia.

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ON THE RATIONALE OF TREATING ALLERGIC DISEASES WITH BACTERIAL PYROGENS

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THE successful isolation of a pyrogenic polysaccharide from a pseudomonas species has stimulated a great deal of interest in its possible therapeutic applications.

The compound, obtained through tryptic digestion from cultures of the organism, has been sufficiently identified by chemical analysis to permit the preparation of solutions of uniform potency.[1] It has been offered to the medical profession under the name of Pyromen by Baxter Laboratories, Morton Grove, Illinois. Its activity can be measured either by induction of fever or by a rise in the number of circulating leukocytes following intravenous administration. The toxicity of the compound is low. The sensitivity of different experimental animals to its various effects differs widely.[2]

In recent years attention has been focused on the mediation of nonspecific effects of infection. The role of the hypothalamus, the anterior pituitary, and the adrenal cortex has been studied by numerous investigators. ACTH and adrenal corticosteroids produce some changes which are similar to those seen after infection, such as an increase in the number of circulating neutrophils, a depression of the number of circulating eosinophils; yet, while infection encourages fever, fever is abolished by ACTH and adrenal corticosteroids. In other words, the similarity between infection on one hand, and administration of ACTH and adrenal corticosteroids on the other, is limited. In fact, Soylemezoglu and Wells[3] showed clearly that, in dogs, ". . . pyrogen produces its characteristic changes in the W.B.C. by mechanisms other than pituitary-adrenal discharge."

In view of the changes in the circulating white blood cells produced by Pyromen, it seemed natural that many of the early investigators spoke about an "ACTH-like effect" of the compound.[4, 5, 6] Consequently, it has been recommended for therapeutic use in a large number of clinical conditions known to respond to the administration of ACTH and adrenal corticosteroids. It is the purpose of this review to summarize experimental and clinical evidence for the usefulness of Pyromen in allergic diseases.

Allergic diseases consist of two well-distinguished phases: (1) a latent immunologic phase which includes exposure to an antigen followed by development and distribution of antibodies, and (2) a manifest "clinical" phase characterized by symptoms which are the result of antigen-antibody union in "shock tissues." The nature of shock tissue has never been adequately explained; not all tissues which carry antibodies are shock tissues. In ragweed sensitivity of man, for instance, reagins are commonly found in the skin, yet only rarely does the skin participate in clinical manifestations of ragweed sensitivity.

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This study has been supported by a grant and a generous supply of Pyromen and Pyromen-placebo from Baxter Laboratories, Morton Grove, Ill.

Increasing evidence suggests that the ground substance of a sensitized tissue plays an important role in determining extent and severity of clinical symptoms.[7] ACTH and adrenal corticoids might exert their beneficial effect by restoring the normal physical and chemical function of impaired ground substance.

It can thus be readily seen that a polysaccharide such as Pyromen might affect allergic diseases in several ways. It might be antigenic in itself. It might alter antibody formation and distribution of antibodies. It might act on shock tissues either by stimulation of existing pituitary and adrenal regulations, or by direct effect on structures involved in the allergic reaction. During the past two years we have studied the effect of Pyromen on various phases of the allergic reaction. On the basis of our findings in guinea pigs it seemed necessary to re-examine the clinical effectiveness of the compound in patients suffering from allergic diseases.

EXPERIMENTAL PROCEDURE

A. Studies on Experimental Animals.—The details of our studies on guinea pigs will be reported elsewhere.[8] In essence, the experiments were divided into four groups. The study of the first two groups (1 and 2) was designed to determine the effect of Pyromen on antibody formation; of the last two groups (3 and 4), to observe the effect of the polysaccharide on the behavior of the bronchial shock tissue following antigen-antibody union.

1. Pyromen (0.2 μg) was injected subcutaneously every other day in guinea pigs sensitized and re-injected with antigens of known high potency, i.e., egg white and ragweed pollen extract adsorbed on aluminum cream. Pyromen injections were initiated one week prior to the sensitizing dose of the antigen. Sensitizing doses of ragweed extract were given intramuscularly, of egg white intraperitoneally; shocking doses were given intracardially after an interval of twenty-one days. Anaphylactic symptoms and death following re-injection were recorded for Pyromen-treated animals and untreated controls.

2. Pyromen (0.2 μg) was injected subcutaneously every other day in guinea pigs sensitized and re-injected with a weak antigen of known potency, i.e., ragweed pollen extract not adsorbed on aluminum cream. In all other respects, the second group was treated like the first group.

3. A single dose of Pyromen (1.0 μg) was given subcutaneously to guinea pigs sensitized with a strong antigen of known potency, i.e., egg white, four hours prior to intracardial re-injection of the antigen. Anaphylactic symptoms and death following re-injection were recorded for Pyromen-treated animals and untreated controls.

4. Nonsensitized guinea pigs, sensitized guinea pigs and guinea pigs which had survived anaphylactic shock, 24 hours previously, through protection with antihistamine drugs, were exposed to Pyromen by inhalation (8 μg nebulized in a DeVilbiss No. 40 nebulizer over a period of 60 minutes). Nonsensitized guinea pigs and guinea pigs which had survived anaphylactic shock were sacrificed four hours after the end of Pyromen inhalation. Sensitized guinea pigs were re-injected intracardially after protection with antihistamine drugs 30 minutes after the end of the inhalation of Pyromen. They were sacrificed

24 hours later. Death of all animals of this group was produced by intraperitoneal injection of Nembutal. Lungs were removed immediately after death and prepared for histologic study.

Determinations of the number of circulating leukocytes and eosinophils were carried out on all four groups immediately prior to sensitization and to the re-injection of the homologous antigen; in surviving animals 24 and 48 hours after anaphylactic shock. In animals of Groups 1 and 2 total white blood cell counts and eosinophil counts were done, in addition, three times before and four hours after administration of Pyromen during the period of sensitization. In animals of Group 3 total white counts and eosinophil counts were done before and four hours after administration of Pyromen, immediately preceding re-injection of the homologous antigen; in Group 4 immediately after and four hours after inhalation of Pyromen.

*B. Clinical Studies.**—The clinical studies were divided into three groups: (1) a study to determine the antigenicity of Pyromen; (2) a study of the effect of Pyromen on the skin reactivity of ragweed-sensitive patients; and (3) a study of the clinical effectiveness of Pyromen in the treatment of allergic disorders of the anaphylactic type, i.e., rhinitis, bronchial asthma, and urticaria.

1. Studies to determine the antigenicity of Pyromen.

Fifty-four patients with various allergic disorders participated in this study. At the beginning of the survey each of the patients was tested by the intradermal injection of approximately 1/100 of a milliliter of a solution containing 10 micrograms of Pyromen per milliliter. Fifty-two of the patients gave negative tests; two patients showed a marked primary reaction—redness and swelling which failed to subside within 60 minutes. The remaining 52 patients were divided into subgroups of 38 and 14 patients each. Thirty-eight patients were given 0.3 c.c. of a solution containing 10 micrograms of Pyromen per milliliter subcutaneously every other day for a period of thirty days. Intradermal tests, using the same solution, were carried out at the end of this period. Fourteen patients were given one single injection of 0.3 c.c. solution containing 10 micrograms of Pyromen per milliliter and re-tested intradermally after an untreated interval of 21 days.

2. Study of the effect of Pyromen on skin tests.

Twelve ragweed-sensitive patients were selected for titration of skin sensitivity before and after administration of 0.3 c.c. of a solution containing 10 micrograms of Pyromen per milliliter subcutaneously every other day for 30 days. Intradermal tests were carried out before treatment and within one hour after the last Pyromen administration in accordance with the procedure outlined by Rappaport and co-workers:[9]

> A freshly prepared extract of giant and short ragweed pollens which contained 50 per cent glycerol for preservation was diluted with normal saline solution for intradermal tests. The first dilution for skin tests contained 0.001 mg. total nitrogen per milliliter. This was diluted at tenfold intervals so that the final or

*The authors are indebted to Dr. Henrietta S. Hartley, Dr. Ruth Rhines, Dr. Alexander Wolf and Mrs. Marjorie T. Hutchinson, the nurse in charge of the Allergy Clinic of the University of Illinois Research and Educational Hospitals, for their assistance in the conduct of the clinical studies.

> sixth dilution was at a concentration of 0.00000001 mg. total N. per milliliter. The 6 dilutions and buffered normal saline solution which served as control were injected intradermally in 2 rows on the volar surface of the forearm. Special care was exercised in attempting to raise uniform wheals about 2 mm. in diameter. Ten minutes after the injection the wheals were examined, outlined with India ink, and transferred to Scotch tape for permanent recording. . . . The wheal sizes and the end points of the reactions recorded on the 2 days were compared in each patient.

3. Forty patients who participated in the clinical evaluation of the effectiveness of Pyromen were selected by the attending staff of the Allergy Clinic of the University of Illinois Research and Educational Hospitals. Of these, 19 had rhinitis, 13 bronchial asthma, and 8 urticaria.

Each of the patients had symptoms of moderate severity—none was intractable; physical examination and allergic survey had been completed prior to the onset of Pyromen therapy. With two exceptions routine treatment was discontinued while Pyromen was administered; symptomatic medication was reduced to a minimum compatible with the comfort of each individual.

Baxter Laboratories prepared Pyromen and Pyromen-Placebo in identical vials distinguished by number. Twenty patients were treated with vials containing Pyromen; twenty with the Pyromen solvent, a solution of 1/6 molar sodium r-lactate. The identity of each vial was kept secret and known only to Baxter Laboratories. It was not made known to us until after completion of the study.

Each patient was given 0.3 c.c. of a solution containing either 10 micrograms of Pyromen per milliliter or of Pyromen-Placebo subcutaneously for a period of 30 days. Name, clinic number, diagnosis, the number of the vial, a brief summary of the clinical response and a final evaluation classified as "improved," "unchanged," or "worse," were entered on cards by the physician in charge of the patient. The study was conducted from mid-March until the end of April, 1952. At the end of the experiment Dr. Robert Herwick, Medical Director of Baxter Laboratories, and one of us (M. S.) met and exchanged information about the contents of the vials and the recorded clinical results.

RESULTS

A. Experimental Studies.—Since we plan to publish the data of our studies on guinea pigs in detail, we would like to present here only a summary of the essential findings:

1. Administration of Pyromen prior to and during sensitization of guinea pigs to a strong antigen fails to decrease the incidence of anaphylactic shock upon re-injection of the antigen.

2. Administration of Pyromen prior to and during sensitization of guinea pigs to a weak antigen appears to increase the incidence of anaphylactic shock upon re-injection of the antigen. Statistical analysis suggests, however, that the number of experimental animals must be increased before valid conclusions can be drawn.

3. Administration of a single large dose of Pyromen four hours prior to re-injection of a potent antigen in highly sensitive guinea pigs fails to reduce

the incidence of anaphylactic shock. Each of the animals so treated showed a marked increase in bleeding tendency four hours after administration of the polysaccharide.

4. (a) Inhalation of Pyromen produces a marked neutrophilic infiltration of the bronchi of normal guinea pigs, with a corresponding decrease of circulating neutrophils. (b) Inhalation of Pyromen by guinea pigs which had a pre-existing bronchial eosinophilia produces a histological picture simulating Loeffler's Syndrome—a marked interstitial pneumonitis. Marked edema prevails; the interstitial spaces are filled with both neutrophils and eosinophils. (c) The inhalation of Pyromen by strongly sensitive guinea pigs 24 hours prior to re-injection of the homologous antigen does not prevent the immigration of eosinophils into the bronchial tissue which occurs as a rule within 24 hours after anaphylactic shock.

B. Clinical Studies.—1. Of 54 patients injected with Pyromen in repeated doses (three times a week, for 30 days) or once (three weeks prior to the test), none developed an anaphylactic sensitivity to the compound. On the other hand, two patients showed a strong primary reaction to the initial injection of the solution. The reaction was found to persist on subsequent injections; consequently, the patients were withdrawn from the experiment.

2. The administration of Pyromen does not alter the skin sensitivity of ragweed-sensitive patients.

3. Of 40 patients treated with Pyromen or Pyromen-Placebo for thirty days, only 38 completed the series of injections. One patient had to be admitted to the hospital because of a miscarriage; one patient had to leave the city unexpectedly. Of 19 patients treated with Pyromen, 8 improved, 7 showed no change, and 4 became worse. Of 19 patients treated with Pyromen-Placebo, 6 were improved, 9 showed no change, and 4 became worse. Of side effects, two patients treated with Pyromen complained about severe headaches without temperature elevation shortly after administration of the compound. The same side reaction was observed in three of the Placebo-treated patients. With this exception, the injections of Pyromen and Pyromen-Placebo were tolerated without untoward reactions.

DISCUSSION

A. Studies on Experimental Animals.—Guinea pigs have been selected for the experimental studies because they respond to sensitization and re-injection with anaphylactic antigens with well-defined and, under optimal conditions, uniform reactions; furthermore, we have used guinea pigs in various studies throughout the past five years and are thus familiar with the inherent variability of the species. Our findings in Groups 1 and 2 of our experimental animals indicate that Pyromen has no demonstrable inhibiting effect on formation and distribution of anaphylactic antibodies; in fact, they furnish suggestive evidence that Pyromen might facilitate sensitization to a weak antigen. In this respect our results are compatible with Stuart's observation that Pyromen, in contrast to cortisone, enhances the Arthus phenomenon in rabbits.[10]

The dose of Pyromen (1.0 μg) administered to highly sensitive guinea pigs four hours prior to re-injection of the homologous antigen is very large. It

was very well tolerated but the animals showed a marked increase in bleeding four hours later when blood was obtained from the ear veins for total white cell counts and eosinophil counts.*

We hesitate to interpret the failure of Pyromen to diminish the incidence of anaphylactic shock as a failure of the compound to act on ground substance of the bronchial mucous membrane, particularly since Friedlaender[12] and co-workers have shown that ACTH administered four hours prior to re-injection of the homologous antigen equally fails to afford guinea pigs protection against anaphylactic shock.

The immigration of polymorphonuclear neutrophils into normal and anaphylactic "eosinophilic" bronchial tissue is compatible with our concepts of the effect of pyrogens. It is interesting, on the other hand, that eosinophilia develops subsequently to anaphylactic shock in bronchial tissue of guinea pigs which have been exposed to the inhalation of Pyromen. Since infection and allergic reaction are, as a rule, mutually exclusive, our findings seem to indicate that the neutrophilia produced by Pyromen is of short duration and can be superseded by the allergic stimulus. The sequence of the changes in the number of circulating leukocytes and eosinophils subsequent to the inhalation of Pyromen makes it likely that they are secondary in nature, mediated through tissue demand and not through pituitary-adrenal mechanisms.

Observations on guinea pigs cannot be applied to man without reservation. We[13] have shown that guinea pigs respond only sluggishly to ACTH and corticoids and that inordinately large doses are required to accomplish physiologic changes comparable to those produced by comparatively much smaller doses in man.

B. Clinical Studies.—1. Observations on 54 patients who received Pyromen subcutaneously, either in one single dose or in repeated doses, made it obvious that the compound must be of low antigenicity or none to man. The primary reaction to the injection of the compound, which occurred in two patients, was persistent; we were able to reproduce the phenomenon on several subsequent occasions. We suspect that the reaction is caused by the solvent rather than by the polysaccharide, but we have not pursued the problem any further.

2. Pyromen does not alter the skin reactivity of ragweed-sensitive patients. Again this observation is of theoretical rather than practical interest, since ACTH fails to alter the skin reactivity even in patients who respond to its administration with considerable relief from clinical symptoms.

3. The evaluation of the effectiveness of drugs in the treatment of allergic manifestations is a difficult task. Allergic diseases have a functional component which often equals or even exceeds the structural changes caused by antigen-antibody union. In extensive tests of the effectiveness of antiasthmatic agents, it was found that patients suffering from severe respiratory disease responded to the administration of placebos of familiar antiasthmatic drugs with marked subjective improvement, even in the absence of objective changes of ventilatory function.[14] It seemed likely that some of the reports on the effectiveness of

* It is interesting that Fitch and Washburne[11] mention "a tendency to increased bleeding on wounding" which occurred in several patients during Pyromen therapy.

Pyromen in the treatment of allergic diseases had failed to recognize the fact that treatment with frequent injections constitutes in itself a therapeutic procedure in patients with predominantly functional changes.

The clinical response of patients suffering from allergic disease of the anaphylactic type (namely, rhinitis, bronchial asthma, and urticaria) to the administration of Pyromen was disappointing. Improvement where reported was not spectacular; in the majority of patients objective tests failed to substantiate subjective claims. The ratio of patients who had improved to those who failed to respond or showed an aggravation of symptoms was almost identical to the ratio produced by the injection of Pyromen-Placebo during the same period. On the basis of our findings, it seems unlikely that Pyromen in subfebrile doses has a rationale in the treatment of allergic disease of the anaphylactic type. We have selected the term "in subfebrile doses" deliberately because it seems possible that Pyromen-produced fever will be of benefit to patients suffering from the limited number of allergic diseases which respond to other types of fever therapy. It is true that other investigators have reported satisfactory results upon the administration of smaller doses of Pyromen than those which have been given to our patients. We are unable to explain this discrepancy.

While intravenous injections of Pyromen, like any intravenous injections, might involve a certain risk, it is our impression that Pyromen can be safely given subcutaneously in the dosage which we have used. Side reactions are few; of those which we have seen, headaches observed in two patients were the outstanding complaint. Interestingly, three patients who received placebo suffered from headaches which were at least as pronounced as those observed in patients who received Pyromen. We do not understand the reason for this peculiar finding, but suspect that an idiosyncrasy to the solvent (1/6 molar sodium r-lactate) rather than to the polysaccharide might be responsible.

SUMMARY

1. Studies of the effect of Pyromen on sensitization and re-injection of guinea pigs with anaphylactic antigens failed to indicate that the compound produces a demonstrable decrease in formation of antibodies or in the results of antigen-antibody reactions.

2. The administration of Pyromen to 38 patients with allergic disease of the anaphylactic type (rhinitis, bronchial asthma, and urticaria) failed to indicate that the clinical effectiveness of the compound is superior to a placebo containing its solvent, 1/6 molar sodium r-lactate.

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THE USE OF A CLOSED SYSTEM IN THE STUDY OF ASTHMA AND EMPHYSEMA

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THE induction of asthmatic attacks under controlled conditions has proved to be a useful and informative method of studying bronchial asthma.[1-8] In this report we have attempted to measure respiratory efficiency as indicated by oxygen saturation and to correlate this with changes in the mechanics of respiration.

The apparatus consists of a combination of units, most of which have been described elsewhere. A closed system in which any desired uniform gas mixture could be administered without rebreathing during uninterrupted recording of respiration was described by Donald and Christie,[9] and again by Gaensler.[10] Aerosols of allergens, histamine and Isuprel, were delivered to the patient from a specially designed compartmented box in a manner less complicated but nevertheless similar to that used by Herxheimer,[11] who studied induced asthmatic attacks in patients breathing oxygen from a Benedict-Roth type of apparatus. The spirometer used by us was especially designed to reduce inertia and friction. Measurement of changes in the oxygen saturation of the arterial blood was made with a Millikan oximeter.[12] Thus changes in the respiratory pattern and the oxygen saturation could be observed simultaneously before, during, and after the induction of an asthmatic attack. Our studies were carried out with the subjects breathing ambient air.

APPARATUS

Two adjacent metal 50 gallon drums were fitted with covers into each of which were welded one central and one off-center iron pipe having an internal bore of one inch (Figs. 1 and 2). To the inside ends of the central pipes were attached neoprene meterologic balloons having a capacity far greater than that of the drums so that the walls would not be stretched when filled with the gas to be inspired. The outside end of each of the four pipes was fitted with a large bore 3 way stopcock. The central pipes of each drum were connected with each other by a large bore hose and the off-center pipes were connected in a similar way. From the opening of a 3-way stopcock placed in the line connecting the two central pipes, a large bore rubber tube carried the gas to be inspired to a specially designed box for the administration of aerosols, and a second tube connected the opposite side of this box with the intake side of a low resistance valve assembly. Expired gas passed from the expiratory side of the valve assembly to a spirometer, the water compartment of which was

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sufficiently large to allow the spirometer bell to move freely without scraping. The bell was constructed of cellulose acetate and, though of conventional size, weighed less than four ounces. Inertia and friction were thereby reduced to a minimum. A fourth tube led the expired gas from the spirometer to the opening of a stopcock placed in the line connecting the two off-center pipes and thence into the space between the metal drums and the balloons. When desired, a soda lime canister could be inserted in the line between the spirometer and this 3-way stopcock. With this arrangement of stopcocks, either balloon could be quickly refilled and the source of gas for the patient could be abruptly changed from one balloon to the other without disturbing either the patient or the respiratory tracing. The type of gas could be likewise abruptly changed if desired. The respiratory excursions were recorded by a pen on moving paper driven by a constant speed kymograph. From a unidirectional wheel driven only by the downward movement of the spirometer bell, a thread passed to a second pen which recorded the minute volume. A fixed pen provided a base line from which measurements were made.

Changes in oxygen saturation were measured with a Millikan oximeter.[12] This apparatus had two channels which permitted simultaneous use of two earpieces.

Experiments were carried out as follows: The subject was comfortably seated and an earpiece of the oximeter was placed on each ear. After maximum dilatation of the capillaries in the ear had occurred (as judged by stabilization of the ear thickness reading) oxygen was given by mask from a demand regulator until no further rise in the saturation reading occurred. At this point the subject was considered to be fully saturated, an assumption that is warranted in the absence of a shunt (other than the bronchial artery) permitting venous blood to reach the pulmonary vein. The light spot of the galvanometer was then set at 100 per cent on the scale and the patient allowed to breath room air until the saturation reading on the galvanometer showed no further change. This reading was considered to be the subject's oxygen saturation while breathing room air. At this point the mouthpiece was inserted, a clip was placed on the nose, the kymograph was started and a tracing of the respiratory pattern was begun. In some experiments the inspiratory capacity, the expiratory reserve volume and the vital capacity were measured before, during, and after an induced asthmatic attack. Saturation readings were recorded at 15 or 30 second intervals for the duration of the experiment, and the ear thickness reading was checked repeatedly. The aerosol was produced with a conventional glass nebulizer supplied with air under pressure flowing at approximately 6 L. per minute. The aerosol passed into one of 2 three-liter compartments in a lucite box, in which there was a vent which could be opened to let air leave as the aerosol entered. The box was so arranged that inspired air could be made to flow through either compartment or through a straight tube by sliding a bar on the top of the box. The density of the aerosol served as a crude index of the amount given, but the amount retained by the subject was not measured, nor was this necessary for the purpose of these experiments.

RESULTS

A total of 11 experiments were carried out with the apparatus described, in four subjects, three of whom had bronchial asthma and one pulmonary emphysema. In each experiment the administration of an allergen or histamine by aerosol caused a temporary decrease in tidal volume, an increase in the functional residual capacity and, in those experiments in which measurements were made, a decrease in the expiratory reserve volume, the inspiratory capacity, and the vital capacity. A decrease in oxygen saturation of the arterial blood, as determined by oximeter, occurred in the severer attacks but was not noted in milder attacks which nevertheless were associated with clear-cut changes in the respiratory pattern as just described.

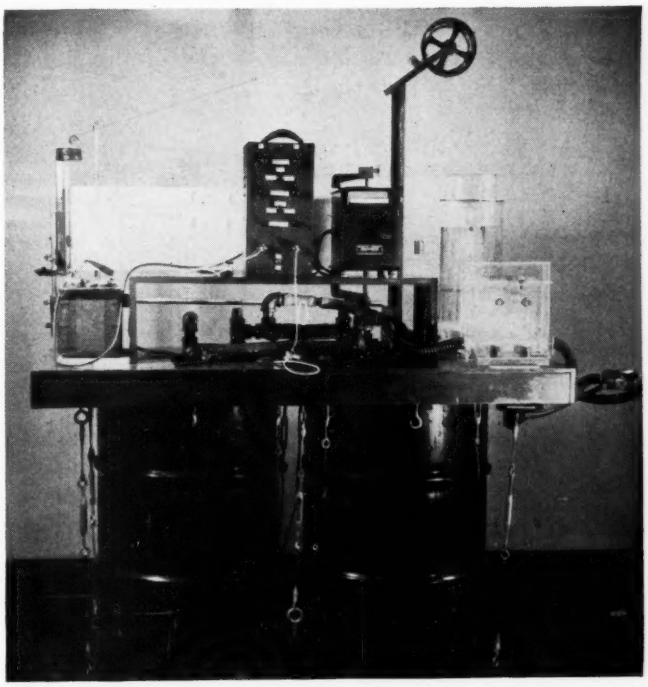

Fig. 1.—Photograph of apparatus described.

Most of the induced attacks were terminated by the administration of an aerosol of Isuprel in the same manner in which the allergen or histamine had been given. In each instance, this caused an increase in the tidal volume and a return of the functional residual capacity toward, but not always to, its original level. After giving Isuprel, the tidal volume always became greater than it had been before the induced attack. This was probably a consequence of the induced difficulty in obtaining oxygen and eliminating carbon dioxide, but it may also have been due to an unrelated effect of Isuprel, for the administration of Isuprel in the same manner to three normal subjects caused hyperventilation in two. One of these subjects had clear-cut systemic effects, including flushing and palpitation. It is possible that the observed effects on respiration in these

two normal subjects receiving Isuprel may have been secondary to cardiovascular effects of the drug. In a like manner, therefore, hyperventilation following Isuprel given to relieve an attack of asthma may be a consequence, in part at least, of its effects on the cardiovascular system. This point requires further study.

The changes in the respiratory pattern and oxygen saturation which accompany a fairly severe attack of asthma induced by the administration of an aerosol of house dust extract* are shown in Fig. 3. This experiment was done with a soda lime canister in the circuit and for this reason the tracing slopes upward due to the removal of carbon dioxide. After a reduction in tidal volume and a rise in functional residual capacity had occurred, the oxygen saturation fell from approximately 94 per cent to 88 per cent. The administration of Isuprel by aerosol three times within two minutes caused a marked increase in ventilation, a rise in oxygen saturation, a lowering of the functional residual capacity and subjective relief of the attack.

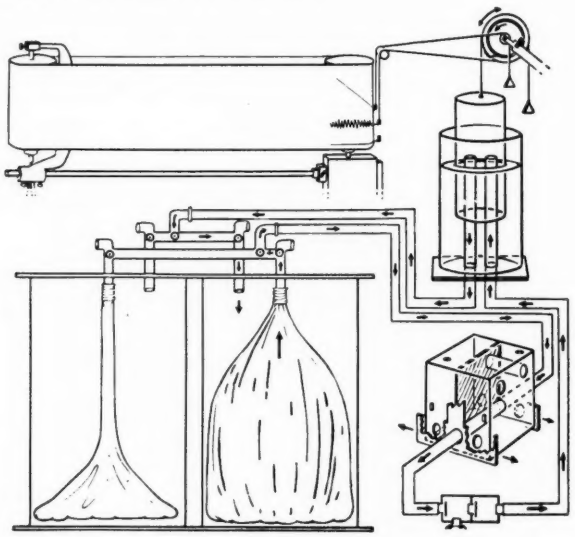

Fig. 2.—Diagram of apparatus described.

A second experiment is shown in Fig. 4. Carbon dioxide was not removed and therefore the tracing is almost horizontal. Upon receiving an aerosol of histamine the subject had a reduction of tidal volume, a rise in functional residual capacity of approximately two liters, and a decrease in the oxygen saturation from 95 per cent to 87 per cent. In this experiment Isuprel was not given. Within a short time the tidal volume increased spontaneously and the minute volume became greater than it had been before the aerosol of histamine was given. However, the oxygen saturation remained low (88 per cent) indicating that the greater minute volume at this time was less effective from the point of view of gas exchange than the smaller minute volume before the induced attack. This difference is probably a result of the increased functional residual

*House Dust Concentrate, Endo Products, Inc. Final Concentration 1:400.

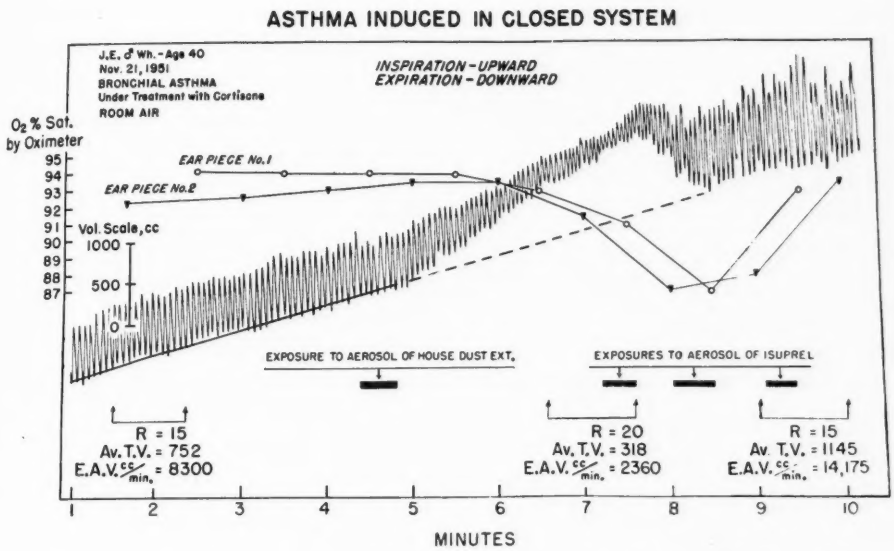

Fig. 3.—*R*, respiratory rate; *Av. T.V.*, average tidal volume in c.c.; *E.A.V. c.c./min.*, effective alveolar ventilation in c.c. per minute calculated by subtracting 200 c.c. (assumed dead space) from the average tidal volume and multiplying by the respiratory rate.

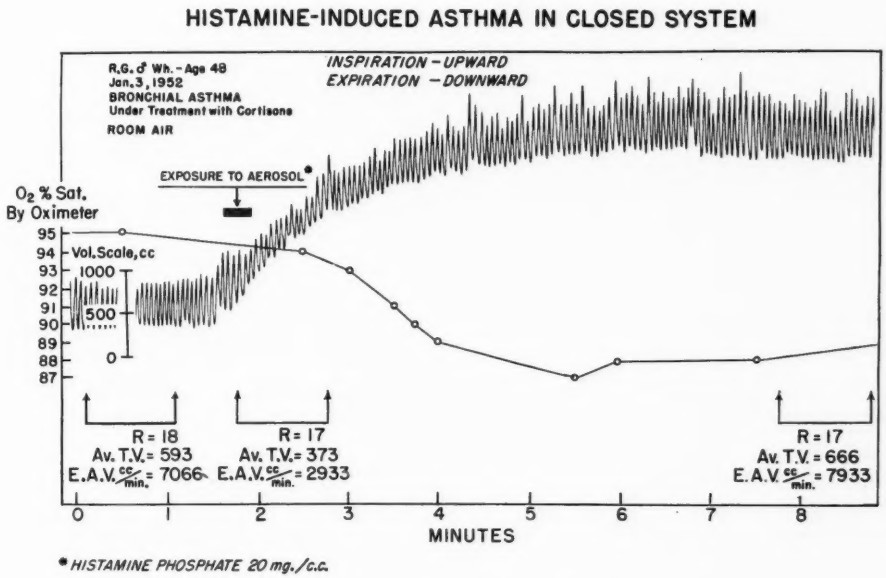

Fig. 4.—For explanation, see Fig. 3.

capacity rather than a change in the ease with which oxygen and carbon dioxide crossed the alveolar membrane. Thus the "effective alveolar ventilation" as usually calculated by multiplying the average tidal volume minus the dead space by the respiratory rate may be misleading during even a mild asthmatic attack. The patient is at a disadvantage, not primarily because of difficulty in getting air into and out of the chest cavity, but more especially because of the inefficient manner in which the air is used. The administration of oxygen by mask and demand regulator at the end of this experiment brought the saturation reading to 100 per cent and as the ear thickness remained constant throughout, the observed changes in oxygen saturation may be considered valid.

A third experiment shown in Fig. 5 illustrates another use of this apparatus in the study of pulmonary disease. A patient with long-standing emphysema first breathed air from one drum and then 100 per cent oxygen from the other while the respiratory rate and depth were continuously recorded. A prompt reduction in the tidal volume and minute volume occurred when oxygen was given and the oxygen saturation which, with the patient breathing air was 90 per cent, rose to 100 per cent. After breathing air for several minutes the tidal and minute volume returned to their former values. This depression of respiration is characteristic of patients with respiratory acidosis when they are given oxygen and the resulting aggravation of the acidosis may be dangerous.[13-17]

DISCUSSION

There are certain advantages in using such a closed system in combination with an oximeter for the study of patients showing disturbances in pulmonary function. A continuous graphic record of the respiratory pattern may be obtained while a patient is exposed to one or more gases, and changes observed in the oxygen saturation, in the respiratory rate, tidal volume, and functional residual capacity, simultaneously. The lung volumes, with the exception of the residual volume, may be measured at will. The effect of an allergen or a drug such as Isuprel upon pulmonary function may also be studied and interpreted with greater accuracy because of the uninterrupted panoramic view of the respiratory pattern.

Certain difficulties which arise with this apparatus have been discussed by others[9] and we have also been concerned with them. The expired air being warm raises the temperature of the system and causes an increase in volume. This effect is temporary, however, as heat loss becomes equal to that derived from the subject within a few minutes. The temporary nature of this effect has been repeatedly observed in the respiratory tracing by us as well as by Donald and Christie.[9] The subject also adds moisture to the system and this may increase the volume. The effect of moisture may be diminished or avoided by having water in the compartments in the drums or by first moistening the air or gas mixture. A third difficulty is the lag of the spirometer bell on inspiration. This is pronounced because the negative pressure must be transmitted through the 200 L. drum and a long air column before the spirometer bell moves. Although decreased by the use of a light plastic bell, the lag on inspiration has not

been eliminated. This lag does not, however, interfere with the accurate recording of the tidal or minute volume during quiet respiration. Furthermore the changes observed are sufficiently large so that the errors introduced by changes in temperature and humidity are unimportant.

In the course of breathing the contents of one balloon, the concentration of carbon dioxide between the wall of the balloon and the drum gradually rises. It was important to learn whether or not diffusion of this gas into the balloon took place and, if so, to what extent. After a ten-minute period of breathing from one of the balloons, analysis showed that carbon dioxide diffused through the balloon wall into the interior but in amounts so small as to have no influence on respiration. For example, after a subject had breathed from one balloon for ten minutes and the contents had been nearly exhausted, analysis of the gas remaining in the balloon showed the concentration of carbon dioxide to be 0.09 per cent. This rose to 0.17 per cent after standing for one hour, and though higher than that which would occur during the course of an experiment, this concentration of carbon dioxide was less than that required to influence respiration.

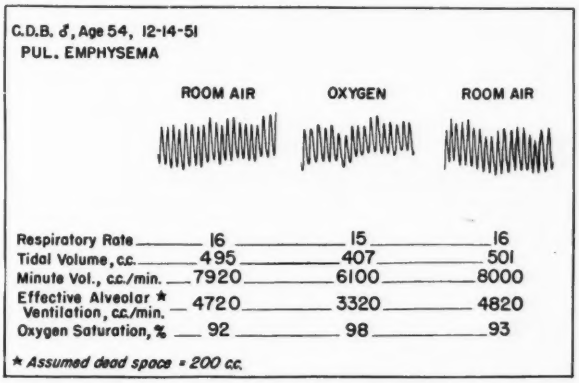
C.D.B. ♂, Age 54, 12-14-51
PUL. EMPHYSEMA
ROOM AIR
OXYGEN
ROOM AIR
Respiratory Rate 16 15 16
Tidal Volume, c.c. 495 407 501
Minute Vol., c.c./min. 7920 6100 8000
Effective Alveolar * Ventilation, c.c./min. 4720 3320 4820
Oxygen Saturation, % 92 98 93
* Assumed dead space = 200 c.c.

Fig. 5.—For explanation, see Fig. 3.

SUMMARY

Experience with an apparatus permitting continuous recording of the respiration is described. The value of simultaneously observing the oxygen saturation with an oximeter is emphasized. The changes which accompany induced asthmatic attacks in subjects breathing ambient air in a closed system included (1) a temporary decrease in tidal volume and (2) an increase in the functional residual capacity. These changes were associated in some instances, with a demonstrable decrease in the efficiency of the lung. Relief of an induced asthmatic attack with aerosolized Isuprel caused an increase in ventilation and a return of the functional residual capacity toward the initial level. Anoxia accompanied severe induced attacks of asthma. The system described was readily adapted to the detection of the decrease in ventilation which follows the inhalation of oxygen by patients with respiratory acidosis.

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INFLUENCE OF ACTH ON ANAPHYLACTIC SHOCK IN GUINEA PIGS

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NOCUOUS agents, capable of producing an alarm reaction, have been found to inhibit under certain conditions the active anaphylactic shock in guinea pigs (Karady et al.[1]). Later, it has been shown (Selye[2]) that this effect is probably due to the discharge of ACTH and that if the hormone is administered one hour prior to the challenging reinjection, the anaphylactic shock is greatly diminished or inhibited.

The problem as to whether anaphylactic reactions can be suppressed or diminished by ACTH or cortisone has since been studied in various experiments. The results, however, are far from being uniform. Some of the investigators observed a certain prevention of fatal shock in guinea pigs (Ørskov,[3] Baschieri,[4] and Simonsen[5]), in mice (Nelson et al.[6]), and especially in adrenalectomized mice after cortisone administration (Dougherty[7]).

On the other hand, a still greater number of experimental workers were not able to demonstrate any influence of ACTH or cortisone on anaphylactic shock in guinea pigs (Léger et al.,[8] Friedlaender and Friedlaender,[9] Harris and Harris,[10] Malkiel,[11] Dews and Code,[12] Dworetzky et al.,[13] and Landau et al.[14]). Furthermore, the inability of ACTH and cortisone to prevent or to diminish the passive Arthus phenomenon has been reported by most of the investigators (Fischel,[15] Germuth et al.,[16] and Harris and Harris[10]). So far, the majority of the experimental findings are in peculiar contrast to clinical experiences and to some other experimental reports in which ACTH and cortisone have been claimed to be effective in preventing systemic allergic inflammations (Berthrong et al.,[17] Rich et al.,[18] and Ebert and Wissler,[19] etc.). The question has therefore arisen as to whether there are allergic reactions, which cannot be influenced by ACTH or cortisone on account of the degree of their intensity or for other reasons.

The fatal contraction of the bronchial muscles in the shock of guinea pigs occurs at a time when morphologic signs of inflammation are virtually absent. Obviously, this mechanism of shock is entirely different from other allergic reactions showing systemic inflammatory changes. The purpose of the present paper is to furnish further evidence of the ability of ACTH to influence this special kind of allergic muscular contraction in vivo.

In our experiments, we employed the active anaphylactic shock. ACTH was given for only a short period before the challenging reinjection in order not to interfere with the formation of antibodies or their quantity in the tissues of the shock organs. As death occurs generally within 2 to 4 minutes after intravenous reinjection, the anaphylactic shock in guinea pigs must be considered an extremely acute stressor agent. Therefore, the adaptive hormone (ACTH) was

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administered in high doses briefly before the reinjection thus concentrating the effect of protection during the short period of shock. Parallel to the first main shock experiment, the nonspecific inflammatory potential was investigated by testing the degree of response to topical irritation (mustard arthritis) (Coutu and Selye[20]) in the animals treated with ACTH and in control animals.

METHODS, ANIMALS, AND MATERIALS

The experiments were carried out in two stages:

In the first, the effect of ACTH on sensitized animals given an approximate LD 50 of antigen in the challenging reinjection was studied. In the second stage, the "line of effect" representing the relationship of the dose of reinjected antigen to the percentage of fatal shock was determined in animals under the influence of ACTH and in controls.

Animals.—Fifty-nine male and female guinea pigs, weighing 240 to 330 grams (mean 295.4 grams), were used in the first experiment; 116 guinea pigs, weighing 230 to 320 grams (mean 267.2 grams), in the second.

Sensitization and Challenging Reinjection.—Active sensitization to horse serum was accomplished by injecting subcutaneously 0.5 ml. of the antigen diluted 1:10 with saline. The challenging reinjection was performed by injecting 0.5 ml. of various antigen-dilutions into the vena femoralis on the twenty-third and twenty-fourth day following sensitization. In the second experiment, the reinjection was given on the twenty-eighth day. A constant time of 10 seconds for the reinjection was observed throughout the experiments.

ACTH.—The dose of ACTH (Connaught) given in both series of experiments was equivalent to 84 mg. Standard La-1-A, per animal, and was administered in the following manner:

ON 3RD DAY BEFORE REINJECTION	ON 2ND DAY BEFORE REINJECTION	ON LAST DAY BEFORE REINJECTION	ON THE DAY OF REINJECTION
18 mg. ACTH (6 inj. of 3 mg. s.c.)	18 mg. ACTH (6 inj. of 3 mg. s.c.)	18 mg. ACTH (6 inj. of 3 mg. s.c.)	10 mg. ACTH i.p. 3 hours before reinjection 10 mg. ACTH i.p. 1 hour before reinjection 10 mg. ACTH i.p. ½ hour before reinjection

In the second experimental series the timing of ACTH injection on the day of reinjection was slightly modified as follows: ten mg. ACTH i.p. 4 hours before reinjection; ten mg. ACTH i.p. 2 hours before reinjection; 10 mg. ACTH i.p. 1½ hours before reinjection. For the calculation of the LD 50 of antigen in the challenging reinjection, the method of Prigge and Schaefer[21] was used.

The "topical irritation arthritis" was induced by para-articular injection of 0.2 ml. of a 10 per cent aqueous mustard suspension into the left hind paw immediately after the last injection of ACTH in the experimental group and simultaneously in the controls. After 3 hours, the difference in the mean diameter of the left and of the right hind paw was measured in millimeters. (For technical details see: Coutu and Selye.[20])

RESULTS

Influence of ACTH on the Quota of Fatal Anaphylactic Shock Induced by a Median Lethal Dose of Reinjected Antigen.—A preliminary study with 8 guinea pigs was undertaken for a rough determination of the LD 50 of antigen for the challenging reinjection. It indicated that this dose was between the dilution 1:2 and 1:8. In order to obtain the LD 50 more exactly, this interval was subdivided logarithmically, and 4 groups of 6 to 7 guinea pigs each were given the following amounts of antigen:

Group I: 0.5 ml., 1:2.64
Group II: 0.5 ml., 1:3.48
Group III: 0.5 ml., 1:4.60
Group IV: 0.5 ml., 1:6.05

The results are shown in Table I. In accordance with these data, we calculated the LD 50 as equivalent to 0.5 ml. of horse serum, diluted 1:3.65.

TABLE I. DETERMINATION OF AN APPROXIMATE LD 50 OF REINJECTED ANTIGEN IN THE FIRST EXPERIMENTAL SERIES

EXPERIMENTAL GROUPS	NO. OF ANIMALS	MEAN BODY WEIGHT AT TIME OF SENSITIZATION (GRAMS)	DOSE OF REINJECTED ANTIGEN (ML.)	NO. OF ANIMALS DEAD IN SHOCK	PERCENTAGE OF FATAL SHOCK
I	7	285.4	0.5 1:2.64	4	57
II	6	291.3	0.5 1:3.48	2	33
III	6	294.5	0.5 1:4.60	4	67
IV	6	297.0	0.5 1:6.05	1	17

On the next day (the twenty-fourth after sensitization), this amount was given to 13 guinea pigs pretreated with ACTH and to 13 control animals.* The results are shown in Table II.

TABLE II. INFLUENCE OF ACTH ON THE QUOTA OF FATAL ANAPHYLACTIC SHOCK INDUCED BY A MEDIAN LETHAL DOSE OF REINJECTED ANTIGEN

EXPERIMENTAL GROUPS	ACTH	NO. OF ANIMALS	MEAN BODY WEIGHT AT TIME OF SENSITIZATION (GRAMS)	MEAN BODY WEIGHT AT TIME OF REINJECTION (GRAMS)	DRY WEIGHT OF ADRENAL GLAND (MG.)	DOSE OF REINJECTED ANTIGEN (ML.)	NO. OF DEAD ANIMALS	FACTOR "P"
I	84 mg. per animal	13	297.7	316.0	83.5 ± 10.0	0.5 1:3.65	5	0.0512
II		13	297.5	336.9	51.9 ± 6.8	0.5 1:3.65	10	

In the control group, 10 of 13 animals died in a typical anaphylactic shock, 2 to 3 min. after reinjection, whereas in the group pretreated with ACTH only 5 animals died. Table II also shows the reduction of body-weight and the enlargement of the adrenal glands in the group treated with ACTH.

*This calculated amount, however, proved to be not a LD 50 but approximately a LD 75 (either the number of animals in each of the 4 groups had been too small to obtain an exact calculation or the sensitivity had increased in the meantime).

The difference in the mortality rates was examined for its significance by means of v. Schelling's t-assay[22] in the following manner:

	DEAD	SURVIVORS	TOTAL
Controls	10	3	13
ACTH	5	8	13
Total	15	11	26

$$t = (10 \cdot 8 - 5 \cdot 3) \cdot \sqrt{\frac{26 - 1}{15 \cdot 11 \cdot 13 \cdot 13}} = 1.95$$

The probability ("P") that the difference in the dead quotas occurred only by chance could therefore be calculated as 0.0512 which was considered to be a significant difference.

In the same group, we were able to demonstrate the reduction of the non-specific "inflammatory potential" under the influence of ACTH by the decrease in response to the topical inflammatory irritation (mustard arthritis); the results are given in Table III.

TABLE III. INFLAMMATORY RESPONSE TO PARA-ARTICULARLY INJECTED MUSTARD SUSPENSION

NO. OF ANIMAL	DEGREE OF INFLAMMATION	
	IN THE ACTH-GROUP	IN THE CONTROL-GROUP
1	0.8	0.9
2	0.2	0.8
3	0.5	1.2
4	0.0	0.9
5	0.6	1.0
6	0.1	0.7
7	0.0	1.1
Mean	0.3 ± 0.12	0.9 ± 0.07
	"P" <0.01	

In order to make a reliable statement concerning the influence of ACTH on the anaphylactic shock in guinea pigs, it was essential to investigate this influence when various doses of antigen were administered in the challenging reinjection; it was necessary to exclude the possibility that there might be antigen dilutions, at which ACTH has no or even an opposite effect; hence we performed a second series of experiments.

Determination of the Relationship Between the Dose of Reinjected Antigen and the Percentage of Fatal Shock in Groups of Animals With and Without the Influence of ACTH.—This time a preliminary experiment with 16 animals indicated a much greater sensitivity of the animals (Table IV). The LD 50 could roughly be evaluated as 0.5 ml. of reinjected antigen, diluted 1:32. In order to administer geometrically increasing doses between the LD 2 and LD 98 in the reinjection, the following amounts of antigen were given to five even-weighing duplicate groups of 20 animals each, half of which were pretreated with ACTH.

Group I: 0.5 ml., 1:8
Group II: 0.5 ml., 1:16
Group III: 0.5 ml., 1:32
Group IV: 0.5 ml., 1:64
Group V: 0.5 ml., 1:128

In each duplicate group the animals pretreated with ACTH and the controls were reinjected simultaneously. The results are shown in Table V and

TABLE IV. DETERMINATION OF AN APPROXIMATE L.D. 50 OF REINJECTED ANTIGEN IN THE SECOND EXPERIMENTAL SERIES

EXPERIMENTAL GROUP	NO. OF ANIMALS	MEAN BODY WEIGHT AT TIME OF SENSITIZATION (GRAMS)	DOSE OF REINJECTED ANTIGEN (ML.)	NO. OF ANIMALS DEAD IN SHOCK	PERCENTAGE OF FATAL SHOCK
I	4	268.0	0.5 1:2	3	75
II	4	268.0	0.5 1:4	1	25
III	4	265.0	0.5 1:8	4	100
IV	4	267.5	0.5 1:16	2	50
V	4	270.0	0.5 1:32	1	25

their graphic pattern in Fig. 1. It can be observed that the lines of effect formed by the groups of animals under the influence of ACTH and by the control groups are parallel, the ACTH-line running on a distinctly lower level than the control line. The results demonstrate in a statistically valid manner the inhibitory effect of ACTH on the anaphylactic shock in guinea pigs for every dose of reinjected antigen between the LD 15 and LD 85. It will be noted that doses above or below these limits would probably show differences between experimental and control groups, which would not suffice to establish the ability of ACTH to influence shock.

DISCUSSION

The "stress" under which the animals try to overcome the fatal asphyxia in anaphylactic shock may suggest that the alarm reaction is involved, and that the already stimulated and enlarged adrenal gland might respond more promptly to an additional stimulation by endogenous adrenocorticotrophic hormone. However, Aguirre and Trigueros[23] failed to show any depletion of ascorbic acid in the adrenal glands, even in fatal anaphylactic shock in guinea pigs. The authors concluded that a hypophyseal-adrenal mechanism cannot be involved in this kind of shock.

It still seems to be uncertain whether the protective effect observed under the influence of ACTH was conditioned by an alarm reaction producing an additional secretion of adrenocorticotrophic hormone and by a more intensive response from an already stimulated adrenal gland. The noxious mechanism of the anaphylactic shock, which caused death within only 2 to 4 minutes in our experiments, is apparently too acute for the endogenous adaptive hormones to become effective.

Simonsen[5] demonstrated a reduction of mortality in anaphylactic shock of guinea pigs and simultaneously a significant fall of the complement under the influence of cortisone. He considered the latter effect to be an explanation for the protection, inasmuch as in the absence of the hemolytic complement, antigen-antibody reactions might become ineffective. However, one of us (Hoene[24]) obtained statistically reliable results, proving the ineffectiveness of various complement-inhibitory substances on passive anaphylaxis in guinea pigs.

TABLE V. INFLUENCE OF ACTH ON THE QUOTA OF FATAL ANAPHYLACTIC SHOCK INDUCED BY VARIOUS DOSES OF REINJECTED ANTIGEN BETWEEN THE L.D. 2 AND L.D. 98

EXPERIMENTAL GROUP		ACTH PER ANIMAL (MG.)	NO. OF ANIMALS	MEAN BODY WEIGHT AT TIME OF SENSITIZATION (GRAMS)	DOSES OF REINJECTED ANTIGEN (ML.)	NO. OF ANIMALS DEAD IN SHOCK	PERCENTAGE OF FATAL SHOCK	TOTAL DEAD ANIMALS	FACTOR "P"
I	a	84	10	262.1	0.5	4	40	in ACTH Groups: 8	<0.01
	b		10	263.0	1:8	8	80		
II	a	84	10	270.0	0.5	3	30		
	b		10	264.0	1:16	6	60		
III	a	84	10	267.9	0.5	1	10		
	b		10	268.2	1:32	3	30		
IV	a	84	9	266.5	0.5	0	0	in Control Groups: 19	
	b		10	263.8	1:64	1	10		
V	a	84	10	267.4	0.5	0	0		
	b		10	264.3	1:128	1	10		

A reduction in the formation of humoral antibodies under treatment with high doses of ACTH or cortisone has been claimed by numerous investigators (Berthrong et al.,[17] Rich et al.,[18] Stoerk and Solotorovsky,[24] Björneboe et al.,[26] Roberts and White,[27] and Germuth et al.[28]). It is possible that the cellular antibodies (responsible for the anaphylactic shock) may also decrease. In our experiments, we have induced the anaphylactic shock at a time when the formation of antibodies and their fixation in the tissues could definitely be considered as accomplished. There is only a remote possibility that the brief treatment with ACTH could have reduced the level of cellular or humoral antibodies, particularly as ACTH or cortisone do not appear to influence the rate of disappearance of antibodies from the sera in passively immunized animals (Fischel et al.,[29] and Germuth et al.[28]).

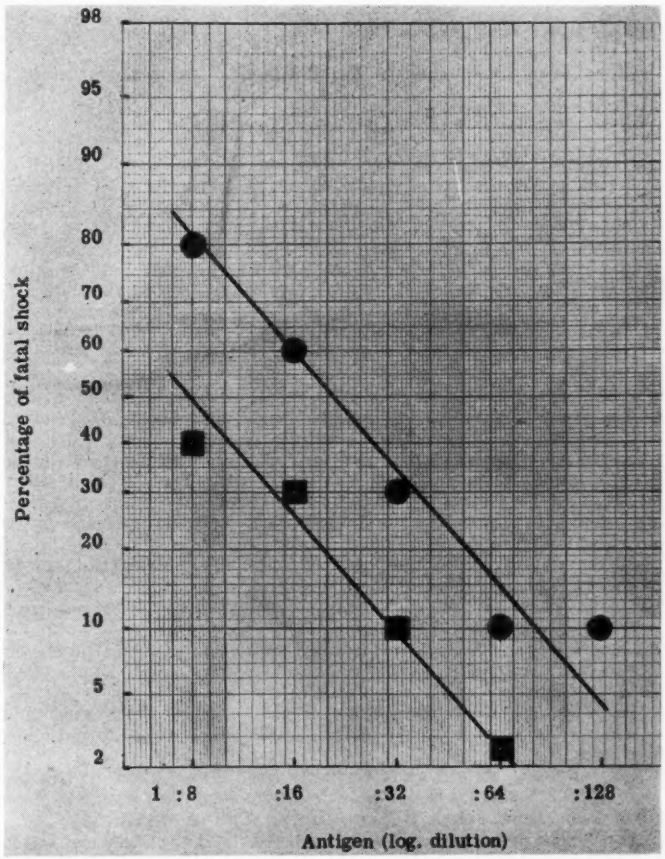

Fig. 1.—Relationship of the dose of reinjected antigen (abscissa) to the percentage of fatal shock (ordinate) for control animals (●———●) and for animals treated with ACTH (■———■).

We consider it possible that the permeability-decreasing effect of ACTH (Robson and Duthie,[30] Benditt et al.,[31] Opsahl,[32] and Ducommun et al.[33]) may have interfered with the antigen-antibody reaction thus reducing the speed with which the intravenously injected antigen could meet the antibodies in the

tissues. But, especially with regard to the well-known catabolic effect of ACTH and cortisone, the shock protection may primarily be mediated by a decrease in the nonspecific reacting power of the shock organs comparable to the decrease in the "inflammatory potential" of tissues under the influence of ACTH or cortisone.

SUMMARY

Heavy overdosage with ACTH, during three days before the challenging reinjection, had a marked inhibitory effect on the active anaphylactic shock induced in guinea pigs by reinjecting antigen at dose levels between the LD 15 and LD 85.

Simultaneously, ACTH effected a significant decrease in the nonspecific "inflammatory potential" as indicated by the response to a para-articularly injected mustard suspension.

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ANAPHYLACTIC SHOCK IN THE PERTUSSIS-VACCINATED MOUSE

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THE resistance of the white mouse to histamine has been referred to as being 1000 times more than that of the guinea pig.[1] Recently, however, it was noted[2] that the sensitivity to histamine could be increased about fiftyfold by a preliminary inoculation with *Hemophilus pertussis* vaccine. This finding has been elaborated and enlarged in subsequent publications.[3-9] Similarly, their resistance to anaphylactic shock has even led earlier workers to indicate that the white mouse is not susceptible. However, although such observations have proved to be erroneous, all agree that shock in the mouse consists of a far less stormy set of sequences than in the guinea pig.[1, 10-12] Mortality figures range as high as 89 per cent[1] apparently depending upon the antigen, number and amount of sensitizing inoculations, sites of injection, time of challenge, size of shocking dose, etc. This refractoriness to histamine has led to the belief[11] that histamine plays no direct or significant role in anaphylactic shock in the mouse as it does in other species.[13] On the other hand, the inconsistency in the production of anaphylactic shock in the mouse as compared to certain other species may be ascribed to its histamine refractoriness.

The technique described by Parfentjev and Goodline[2] by which white mice are made extraordinarily sensitive to histamine has been utilized to restudy the relationship between histamine and anaphylactic shock in this species of animal.

EXPERIMENTAL PROCEDURES AND RESULTS

Production of Sensitivity to Histamine.—The technique of Parfentjev and Goodline[2] was used essentially as described. Commercially available Pertussis vaccine (Lederle) was used throughout. Female, white mice* of about 20 grams in weight were inoculated via the intraperitoneal route (i.p.) with 0.5 ml. of diluted vaccine containing about 18,750 million Phase I organisms. On the fourth day following vaccination, the mice were injected i.p. with 0.5 ml. of a solution of histamine diphosphate in saline. Using this technique, the LD_{50} was found to be approximately 0.8 mg. for a 20 gram mouse. The LD_{50} of histamine diphosphate for unvaccinated mice was approximated at 38 mg. for a 20 gram mouse. Since the number of *H. pertussis* organisms used above for sensitization proved to be highly toxic with the resultant loss of many ani-

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*The female, white mouse has been found to be more sensitive than the male to histamine both before and after vaccination.[14]

mals prior to shocking, the dose per mouse in subsequent experiments was reduced to 8,750 million organisms. The animals thus vaccinated were found to have a sensitivity to histamine comparable to that above.

TABLE I. EFFECT OF VARIOUS VACCINES ON HISTAMINE SUSCEPTIBILITY OF THE FEMALE WHITE MOUSE

VACCINE	MILLION ORGANISMS/0.5 ML. DOSE	MG. HISTAMINE PHOSPHATE/MOUSE	MORTALITY/NO. TESTED
Control		38.0	LD_{50}
H. pertussis	18,750	0.8	LD_{50}
H. pertussis	18,750	1.5	4/4
Brucella abortus	1,200	1.6	20/33
Entoral suspension	15,000	1.0	0/12
N. Catarrhalis	600	0.9	0/10
		1.5	0/10
		3.0	0/10
		10.0	0/5
		20.0	0/5
Typhoid-paratyphoid	700	1.0	0/5
		2.0	0/4
		5.0	0/6
		10.0	0/10
Cholera	1,000	1.6	1/5
H. influenzae	8,750	0.8	0/22
Influenza virus	diluted 1:10	1.6	2/20
	diluted 1:2	1.6	0/20

Vaccine inoculated intraperitoneally. Histamine given intraperitoneally on fourth day.

Effect of Other Vaccines on Histamine Sensitivity.—In order to determine whether this effect was a nonspecific result due to particulate matter, mice were inoculated with a series of vaccines and shocked in a like manner. Table I indicates that *Brucella abortus* vaccine (Sharp and Dohme), 1200 million organisms per mouse, also caused an increase in histamine sensitivity. The amount of histamine diphosphate which gave a mortality rate of 20/33 for *Brucella abortus*-vaccinated mice was 2 times the LD_{50} dose for pertussis-vaccinated mice. The mortality rate was determined as the number of animals dead within twenty-four hours/number of animals injected with histamine. Within the range of the amount of histamine used, no other vaccine caused any significant increase in histamine sensitivity.

TABLE II. EFFECT OF VARIOUS VACCINES ON ANAPHYLACTIC SENSITIVITY OF THE HORSE SERUM-SENSITIVE FEMALE WHITE MOUSE

VACCINE	MILLION ORGANISMS/0.5 ML. DOSE	MORTALITY/NO. TESTED
Control	——	3/66
H. pertussis	8,750	95/102
Brucella abortus	1,200	7/16
Typhoid-paratyphoid	700	1/15
Cholera	1,000	1/18
H. influenzae	8,750	1/12

Mice sensitized with a single intraperitoneal injection of 0.03 ml. horse serum. Challenge dose of 0.1 ml. horse serum given intravenously on fifteenth day.

Enhancement of Anaphylactic Shock by H. pertussis Vaccination.—Mice were inoculated i.p. with 0.5 ml. of a mixture containing 8,750 million *H. per-*

tussis organisms and 0.03 ml. horse serum. On the fourth subsequent day a series tested with histamine diphosphate showed them to be markedly sensitive. Fifteen days after sensitization the mice were challenged by the intravenous (i.v.) administration of 0.1 ml. of horse serum. A control group of animals, sensitized to horse serum as above but not receiving the *H. pertussis* vaccine, showed a mortality rate of 3/66 as compared to 95/102 for the vaccinated mice. With respect to histamine sensitivity after fifteen days *H. pertussis*-vaccinated mice still showed a mortality of 4/4 when injected with 1 mg. and 1/4 with 0.5 mg. of histamine diphosphate.

Effect of Other Organisms on Anaphylactic Shock.—Of several vaccines tested in an identical manner for their enhancement of anaphylactic sensitivity, only *Brucella abortus* vaccine, 1200 million organisms per mouse, produced any significant increase in mortality rate under comparable experimental conditions, as indicated in Table II.

Effect of Pyribenzamine on Anaphylactic Shock.—Mice were sensitized to pertussis vaccine and immunized to horse serum by the technique previously described. On the fifteenth day, the mice were pretreated with varying concentrations of Pyribenzamine hydrochloride fifteen minutes prior to the intravenous challenge dose of horse serum. The Pyribenzamine was in aqueous solution: 0.5 ml. doses were given intraperitoneally. The results shown in Table III indicate that there was no protection against anaphylactic shock in mice receiving 0.5, 0.25 or 0.2 mg. of the drug per mouse.

The Relationship Between Histamine and Anaphylactic Shock.—Mice were immunized by the intraperitoneal injection of 0.03 ml. of horse serum. On the eleventh subsequent day, the animals were sensitized to *H. pertussis* as previously. On the fifteenth day post immunization (fourth day post pertussis sensitization), the animals were divided into two groups. Group 1 was challenged by an intraperitoneal injection of approximately a LD_{50} of histamine diphosphate. Seven deaths were recorded out of ten animals tested. These mice were thus shown to be markedly histamine sensitive. Group 2 was challenged with 0.1 ml. horse serum given intravenously. Table IV shows the results; a mortality of only 7/64 was observed. Therefore, in spite of the fact that the mice exhibited an extraordinary sensitivity to histamine, the mortality rate as a result of anaphylactic challenge did not differ significantly from that observed for the control group given only a sensitizing dose of horse serum.

In the following experiment the constituent antigens were administered on the same day but separately in different sites, vaccine at the usual dosage level, i.p., and horse serum, 0.03 ml., subcutaneously. Challenge with horse serum was performed, as usual, on the fifteenth day. The results, as indicated in Table IV, show a mortality of 9/18. The above experiment was carried out as described, i.e., vaccine given intraperitoneally, and not vice versa because it had been observed that subcutaneous vaccination failed to markedly enhance histamine sensitivity. Such mice showed a mortality of only 2/19 on the fourth day when challenged with histamine in the usual manner and a repeat challenge on the eighth day gave 0/17 deaths.

TABLE III. EFFECT OF PYRIBENZAMINE IN ALTERING ANAPHYLAXIS IN THE FEMALE WHITE MOUSE

PYRIBENZAMINE DOSE/MOUSE	MORTALITY/NO. TESTED
Control	95/102
0.5 mg.	31/37
0.25 mg.	16/17
0.2 mg.	9/10

H. pertussis, 8,750 million organisms, and horse serum, 0.03 ml., injected intraperitoneally. Challenge by 0.1 ml. horse serum intravenously on fifteenth day. Pyribenzamine given intraperitoneally fifteen minutes before challenge.

DISCUSSION

The action of *H. pertussis* in markedly increasing anaphylactic sensitivity appears to be specific for that organism, although, of the many vaccines studied, that of *Bruccella abortus* did enhance both histamine and anaphylactic sensitivity to a small degree. This observed parallelism between enhancement of histamine and anaphylactic sensitivity offered the opportunity to re-evaluate their relationship in the mouse.

The suggestion has been advanced[11] that histamine plays no direct or significant role in mouse anaphylaxis. It should be noted that mice dying of anaphylactic or even histamine shock do not die a respiratory death due to bronchial constriction as do guinea pigs. Since the antigenic challenge of anaphylatically-sensitive mice seems to be so uniformly and reproduceably fatal, histamine, if it is of major importance in the causation of death, is being liberated in relatively large quantities.

It seemed that one way in which to test the histamine theory was by the use of antihistamines. The antihistaminic compounds are regarded as agents which are specific histamine antagonists in histamine and anaphylactic shock in most species.[15] The demonstration that an antihistamine can modify anaphylactic shock in the mouse might lend support to the suggestion that histamine plays more than a negligible role in mouse anaphylaxis. Moreover, it has been observed[2, 4-6, 28] that certain of these antihistamines, Bromothen, Thephorin, and Phenergan and Chlortrimeton effectively prevent the histamine shock in vaccinated mice. Our data for Pyribenzamine which follow (Table V) corroborate these findings. However, we have noted that Pyribenzamine in a single dose fifteen minutes before i.v. challenge with the homologous antigen is without any appreciable effect in protecting actively sensitized and pertussis-vaccinated mice against the anaphylactic shock. The suggestion that

TABLE IV. EFFECT OF VARIOUS INOCULATING SITES AND TIMES ON ANAPHYLACTIC SENSITIVITY IN THE FEMALE WHITE MOUSE

	HORSE SERUM 0.03 ML.	H. PERTUSSIS VACCINE 8,750 MILLION ORGANISMS		CHALLENGE DOSE 0.1 ML. HORSE SERUM GIVEN	
SITE	DAY OF EXPERIMENT	SITE	DAY OF EXPERIMENT	INTRAVENOUSLY ON FIFTEENTH DAY	MORTALITY/NO. TESTED
I.P.	1	—	—		3/66
I.P.	1	I.P.	1		95/102
I.P.	1	I.P.	11		7/64*
S.C.	1	I.P.	1		9/18

*Challenge of this group with histamine diphosphate, 1 mg. i.p., gave a mortality of 7/10.

TABLE V. EFFECT OF PYRIBENZAMINE ON THE HISTAMINE SHOCK OF PERTUSSIS-SENSITIZED MICE

MG. PBZ/MOUSE	MORTALITY
—	7/15
0.5	0/10
0.25	1/20
0.10	3/20
0.05	4/20
0.01	9/20

Histamine phosphate, 1 mg. per mouse, given on the fourth day.

pertussis-vaccinated and horse serum-sensitive mice show a high anaphylactic mortality rate because they are capable of releasing large quantities of histamine seems to have little basis at least by these experiments. Thephorin was shown by Parfentjev[4] to exert a marked influence on the anaphylactic shock produced by the intravenous injection of *H. pertussis* antigen into pertussis-sensitized mice.

Further indication that histamine is not significant in the anaphylactic phenomenon stems from the observation that mice, immunized to horse serum and subsequently made acutely sensitive to histamine by *H. pertussis* vaccination, showed only a relatively low mortality rate (7/64) as compared to the extreme results obtained in (a) horse serum-immunized mice (3/66) and (b) simultaneously immunized and vaccinated controls (95/102). The possibility does exist that this organism possesses the peculiar faculty of acting as a "schlepper" or adjuvant with the enhancement of the antigenic capacity of horse serum. Of the vaccines utilized in the "mixed" technique, only *H. pertussis* and *Brucella abortus,* the latter to a much less marked degree, enhanced the anaphylactic sensitivity. This capacity seems to be specific or else other particulate matter, e.g., organisms, other cells, viruses, India ink, trypan blue, etc., would be expected to do likewise. None of these materials produced this effect. However, the observed increase in sensitivity of the vaccinated mouse to histamine seems difficult to explain on the basis of a "schlepper" effect. That the "schlepper" effect may be necessary to some degree can be seen from the results obtained when *H. pertussis* is given intraperitoneally and horse serum injected subcutaneously but given on the same day. The degree of mortality is low (9/18). It is known, however, that protein administered subcutaneously is a far less effective antigen than the same protein given intraperitoneally. Vaccination, apparently then, has the effect of "preparing" the mouse for the further insult of anaphylactic challenge.

Of current interest[16] is the observation that an inadvertant association seems to exist between the clinical syndromes of limb paralysis and prophylactic immunization therapy. Some of the accidents reported in the literature[17-24] have specifically occurred following the vaccination of children with *H. pertussis* or pertussis-containing mixed vaccines. The number of clinical cases reported at the present time is too few and proper control is lacking for any significant evaluation; nevertheless, an important approach to the question of the relationship between immunization and clinical paralysis, should such eventually be found to be true, is the observation that, experimentally, pertussis

vaccination leads to a marked increase in anaphylactic sensitivity in white mice when the antigen is mixed with the vaccine and simultaneously injected. It must be emphasized that any resemblance between the increase in anaphylactic death produced experimentally and the clinical syndrome of paralysis following vaccination with *H. pertussis* is only suggestive and conjectural at the present time. A report[27] has also appeared which describes fatal anaphylactic shock in the human being following a second injection of a mixed pertussis-diphtheria toxoid vaccine. Our present interest concerns itself with a study of the experimental production of an increased anaphylactic sensitivity.

The mechanism involved in the production of histamine and anaphylactic sensitivity is markedly intriguing and demands further studies. Essentially, no approach has been made except that it has been reported[8, 25] and verified by us that cortisone is effective in preventing histamine shock in pertussis-sensitized mice. We have noted that cortisone also alters the mortality of the anaphylactic shock in the sensitized mouse as it has been reported for the unsensitized animal.[26]

SUMMARY

1. It has been confirmed that *H. pertussis* vaccination of white mice produces a fiftyfold increase in histamine sensitivity.

2. *Brucella abortus* vaccine enhanced histamine sensitivity to a much less but significant degree.

3. Mice immunized by an intraperitoneal injection of a mixture of *H. pertussis* vaccine and horse serum become markedly anaphylactically sensitive to the serum. *Brucella abortus* vaccine has a somewhat similar effect on anaphylaxis.

4. The results of further experimentation seem to substantiate the impression that histamine plays a minor role in anaphylaxis in the mouse even when the mouse has been made markedly histamine sensitive.

5. The possible relationship between the enhancement of experimental anaphylaxis by *H. pertussis* vaccine and the questionable observation of the production of the clinical syndrome of paralysis following vaccination is discussed.

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PHARMACOLOGIC AND ANAPHYLACTOGENIC PROPERTIES OF STAPHYLOCOCCUS PROTEIN AND UNFRACTIONATED EXTRACT

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A STUDY of the cutaneous reactions to staphylococcus extract and various protein and polysaccharide fractions has been previously reported from this laboratory.[1] We are now reporting preliminary studies on the pharmacologic and anaphylactogenic properties of a whole unfractionated extract and two protein fractions of staphylococcus.

Previous workers have utilized, for the most part, culture filtrates which vary considerably with methods of culture, and which may contain interfering substances.[2] In the present work, which is part of a study of the role of staphylococcus in bacterial hypersensitivity, we have employed certain chemical fractionated derivatives and whole extracts from ground washed bacteria.

MATERIALS AND METHODS

Two strains of staphylococcus, one pathogenic (F-10) and one nonpathogenic (F-7) were the sources of the material used in this study.[1] In order to eliminate potential antigens from the media as far as possible, the whole unfractionated extract, consisting of an aqueous suspension of the lyophilized and ground, washed bacterial mass was prepared from a 48 to 72 hour culture grown in a synthetic media, modified from Bernheimer and associates,[3] and passed through a Seitz filter. From the whole extract one of the two protein fractions was precipitated at pH 3.5. This actually consists of one fraction precipitable at pH 5.3 and one precipitated at pH 3.5, referred to as Fraction A-1 and A-2 in a previous report.[1] Since these fractions demonstrated cross reactivity on precipitin tests,[1] they were combined in the present work and referred to, as fraction A. The second protein fraction, referred to as fraction B, was precipitated with trichloracetic acid after removal of fraction A. All fractions were dialyzed against a bicarbonate buffer, pH 8.0, before use.

The whole extracts and protein fractions of both F-10 and F-7 strains of staphylococcus were investigated with regard to their pharmacologic effects upon normal guinea pigs and isolated suspended segments of guinea pig ileum. Investigations of anaphylactogenic properties were undertaken on animals which had received one to three months previously, a series of injections of the whole extracts and fractions in an attempt to bring about sensitization. Extracts and fractions were also studied from the standpoint of heat stability.

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Sensitization to the whole extract and protein fractions of strains F-10 and F-7 was attempted in 21 male guinea pigs each weighing between 250 and 300 grams. Four animals received whole extract (F-10), 5 animals received fraction A (F-10) while 4 animals received fraction B (F-10) in four consecutive daily intradermal injections totaling 1.0 mg. of phosphotungstic acid precipitable nitrogen, hereafter termed "protein nitrogen."

Three animals received 1.0 mg. protein nitrogen of whole extract (F-7) intravenously, followed by three daily intradermal injections of 0.1 mg., totaling 1.3 mg.

Three animals received 0.7 to 2.5 mg. protein nitrogen of fraction A (F-7) intravenously, followed by three consecutive daily injections of 0.13 mg. intradermally, totaling 1.0 to 3.0 mg.

Two animals received fraction B (F-7) in four consecutive daily intradermal injections totaling 0.24 mg. of protein nitrogen.

The initial intravenous injections in groups receiving whole extract (F-7) and fraction A (F-7), respectively, were given to study the effect, if any, of these materials in normal animals. Since there was no effect, subsequent intradermal injections were given in an attempt to bring about sensitization.

To test for anaphylaxis, four to twelve weeks after the last sensitizing injection of extracts or fractions, challenging injections of 0.1 to 0.7 mg. protein nitrogen of the corresponding materials were administered intravenously or intracardiac.

To test for primary toxicity, 19 normal, unsensitized animals received intravenous, intracardiac, or intraperitoneal injections of the extracts or fractions in amounts from 2 to 6 times that employed in the challenging injections in the "sensitized" animals. This group of controls included the groups sensitized to whole extract (F-7) and fraction A (F-7) in which the first sensitizing doses were large intravenous injections.

Autopsies were done on both normal and sensitized animals immediately after death. When recovery seemed probable, usually in 15 to 60 minutes, the animals were killed by a blow on the head.

Tests on isolated guinea pig ileum were made by suspending 1 to 2 inch segments in a 3 ml. bath containing Tyrode solution maintained at 37° C. A given volume of extract or fraction (usually 0.2 or 0.4 ml.) containing 0.02 to 0.06 mg. protein nitrogen was added to the bath and reactions of the segment were recorded on a moving smoked drum. Repeated additions to the bath of the same or other materials were similarly studied with each suspended segment of ileum, the segment being washed three times between each addition. Forty-six segments from 12 normal animals and 83 segments from 14 sensitized animals were tested. The materials were added to the bath in various sequences with different segments from each animal. The criteria for a specific anaphylactic reaction to a given material were: (1) initial contact followed by contraction of the segment within one minute, (2) subsequent contacts associated with loss of reactivity (desensitization), (3) contraction of the segment to histamine after desensitization, and (4) absence of contraction to the material by segments from normal (nonsensitized) animals.

RESULTS

Results of chemical analysis of the fractions are shown in Table I. The presence of purine bases and relatively high concentrations of carbohydrate and organic phosphorus in the A fractions, as well as their insolubility in dilute acids, suggest that these fractions contain nucleoprotein. In contrast, the B fractions contain no purine bases and relatively little carbohydrate or phosphorus, and are completely soluble in either dilute or concentrated alkali or acid. None of the extracts or fractions possessed histamine-like activity as tested on the isolated suspended guinea pig ileum in that contractions did not consistently follow repeated contact with these materials.

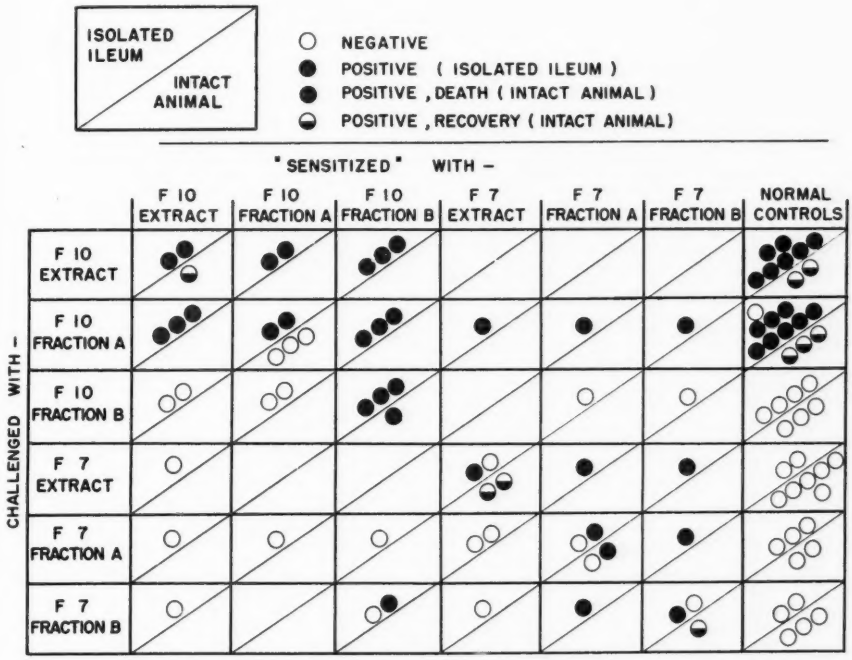

Fig. 1.—Effect of unfractionated extracts and fractions of pathogenic (F-10) and nonpathogenic (F-7) strains of staphylococcus on normal and sensitized guinea pigs and isolated segments of guinea pig ileum. Within each box each symbol represents a separate animal. Positive reactions were anaphylactic or anaphylactoid, as discussed in text.

Pharmacologic Effects of Extracts and Fractions.—Whole extract or fraction A of the pathogenic strain (F-10) injected intracardiac or intraperitoneally into five normal animals was followed by moderate to severe anaphylactoid reactions, characterized by cough, retching, dyspnea and varying degrees of shock (Fig. 1). All of the animals were recovering, when killed within 15 to 60 minutes. Autopsy revealed moderate to marked pulmonary emphysema in all cases. In contrast, neither fraction B (F-10), whole extract (F-7), fraction A (F-7) nor fraction B (F-7), injected intracardiac or intravenously, produced more than an occasional equivocal symptom and autopsies revealed little or no pulmonary emphysema.

The whole extract of the pathogenic (F-10) strain, when placed in contact with the normal segment of ileum, caused a maximal contraction within about 30 seconds, which persisted for about a minute, after which time the segment relaxed and the tracing returned to the base line (Fig. 3). After washing, subsequent additions of this extract to the bath resulted in progressively diminishing and finally absent contraction. After "desensitization" the segment still reacted normally to histamine. Fraction A (F-10) possessed the identical activity of the whole extract (Fig. 2). This reaction occurred with consistency in both control and sensitized animals (Fig. 1). In addition, "desensitization" to whole extract resulted in loss of reactivity to fraction A (F-10), and conversely "desensitization" to fraction A (F-10) caused loss of reactivity to the whole extract. The reactions produced by the whole extract (F-10) and fraction A (F-10) are similar to those produced by snake venoms[4, 5] and other toxins.[6, 7] This toxic effect was not present in fraction B (F-10), nor was it found in extract or fractions of the nonpathogenic (F-7) strain (Fig. 1). This toxic factor as demonstrated on the isolated suspended segment of ileum from normal animals was heat stable, withstanding boiling for 15 minutes.

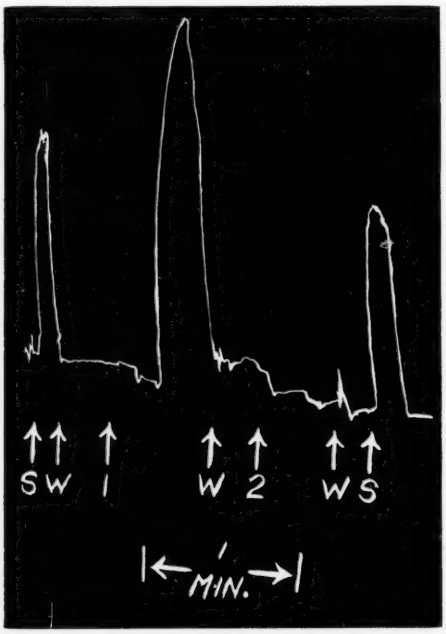

Fig. 2.—Effect of fraction A of pathogenic strain (F-10) upon isolated suspended segment of ileum from a normal guinea pig. S indicates addition of 0.04 μg. of standard histamine base; W indicates 3 washes.

Arrow 1, 0.025 mg. P.N. fraction A (F-10) added to bath. Maximal contraction after 30 seconds.

Arrow 2, 0.05 mg. P.N. fraction A (F-10) added to bath. No contraction in one minute (desensitization).

Therefore, though the number of animals is small, it would seem that the whole extract and fraction A (F-10) of the pathogenic staphylococcus has an anaphylactoid effect both in the intact animal and the isolated ileum of normal guinea pigs.

TABLE I. ANALYSIS OF DIALYZED PROTEIN FRACTIONS OF PATHOGENIC (F-10) AND NONPATHOGENIC (F-7) STRAINS OF STAPHYLOCOCCUS

	A (F-10)	B (F-10)	A (F-7)	B (F-7)
Total nitrogen[a]	12.54	12.35	13.71	10.00
"Protein" nitrogen[b]	10.64	12.13	13.30	10.00
Total phosphorus[c]	3.68	1.26	2.10	0.00
Inorganic phosphorus[c]	0.12	0.00	0.00	0.00
Carbohydrate (as pentose or glucose)[d]	14.70	7.80	10.80	1.47
Purine bases (qualitative)[e]	+	0	+	0

[a]Micro Kjeldahl n; Kabat and Meyer: Experimental Immunochemistry.

[b]Phosphotungstic Acid precipitated N—Modified from Cooke, Allergy in Theory and Practice.

[c]Fiske-Subbarow: J. Biol. Chem. **66**: 375. 1925.

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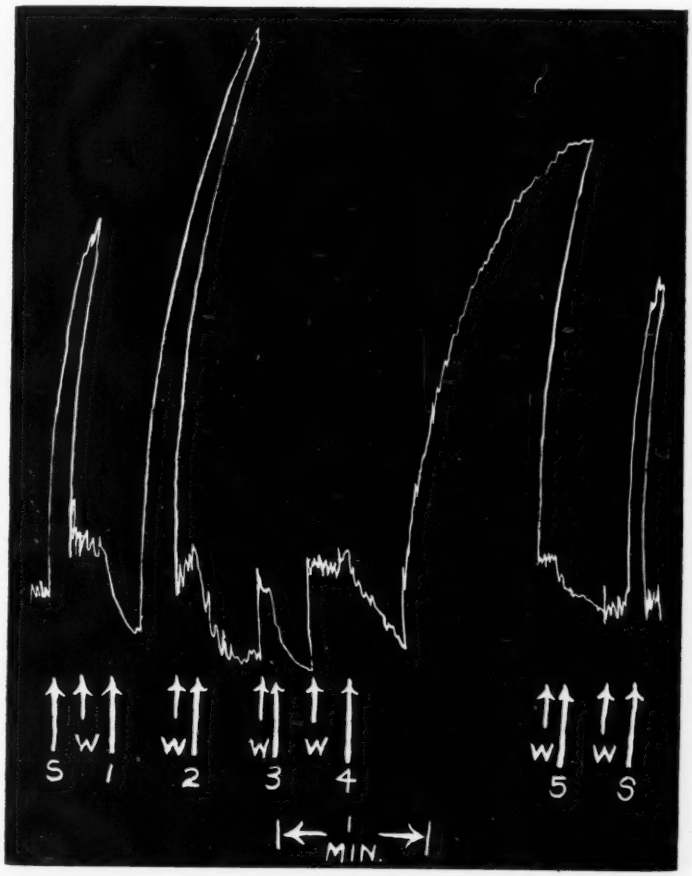

Fig. 3.—Effect of unfractionated extract and fraction B of the pathogenic strain (F-10) of staphylococcus upon isolated ileum from guinea pig sensitized to fraction B (F-10). *S* indicates addition of 0.04 μg of standard histamine base, *W* indicates 3 washes.

Arrow 1, 0.025 mg. P.N. extract (F-10); maximal contraction after 30 seconds.

Arrows 2 and 3, 0.05 mg. P.N. extract (F-10). No contraction in one minute (desensitization to extract F-10).

Arrow 4, 0.028 mg. P.N. fraction B (F-10). Maximal contraction after 30 seconds.

Arrow 5, 0.056 mg. P.N. of fraction B (F-10). No contraction in one minute (desensitization).

Anaphylactogenic Effects of Extracts and Fractions.—Anaphylaxis was demonstrated either in the intact animal or isolated ileum or both with whole extracts and all fractions from both pathogenic and nonpathogenic strains (Figs. 1 and 3). However, since whole extract (F-10) and fraction A (F-10) produced anaphylactoid reactions in the control animals, true anaphylaxis cannot be claimed for these two groups. The three animals sensitized and challenged with fraction A (F-10) showed no reactions, whereas this fraction produced anaphylactoid symptoms in three control animals. A few cross reactions occurred, concerning which no conclusions are warranted until further studies are done.

Anaphylaxis demonstrated in the isolated, suspended ileum of guinea pigs sensitized with the nontoxic extract or fractions was unaffected by previous "desensitization" to the toxic extract or fraction A (F-10) (Fig. 3). However, the anaphylactoid reaction to whole extract (F-10) or fraction A (F-10) was frequently lost following desenitization with specific antigen in segments from sensitized animals.

SUMMARY AND CONCLUSIONS

The pharmacologic and anaphylactogenic effects of extracts and two protein fractions of staphylococcus in guinea pigs and isolated segments of guinea pig ileum have been studied.

The unfractionated extract of a pathogenic strain of staphylococcus produced anaphylactoid reactions in both the intact animal and the isolated ileum. This toxic activity was limited to one of the two protein fractions and was heat stable.

Neither extract nor fractions of the nonpathogenic strain of staphylococcus demonstrated toxic activity.

Since extracts and fractions were obtained from organisms washed free of filtrate, it is concluded that the toxic factor is somatic in origin. Results of chemical analysis suggest that the toxic protein fraction contains nucleoprotein.

Anaphylaxis was demonstrated with all extracts and fractions of both strains of staphylococcus with the exception of the unfractionated extract and the toxic protein fraction of the pathogenic strain. True anaphylaxis could not be proved with these latter two materials because of their primary toxicity. This toxic factor, which may mask anaphylaxis, must be taken into consideration in any study of hypersensitivity to this organism.

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A CLINICAL STUDY OF PHENACETIN-CAFFEINE IN THE TREATMENT OF HAY FEVER

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ALTHOUGH long used as adjuvant drugs in analgesic compounds, Phenacetin (acetophenetidin), and caffeine have recently achieved great popularity when used together with antihistamine compounds as proprietary "cold" and hay fever remedies. While antihistamines are of undoubted value in hay fever, the adjuvant value of Phenacetin and caffeine in this condition remains speculative. We have been able to find no reports on this subject in the literature of the last ten years. Of course, caffeine may be used in these preparations more as a corrective for the side effects of the antihistamines than as an adjuvant to them, but the basis for the inclusion of Phenacetin is not well established.

Recently the effect of two antihistamine preparations on a group of patients with ragweed hay fever was studied in the allergy clinics of the University Hospital and University Health Service. One of these preparations was Thenylpyramine Hydrochloride (30 mg.) and the other was the same in combination with Phenacetin (200 mg.) and caffeine (30 mg.). In this study, two control medications were used: a glucose placebo and a tablet containing 200 mg. of Phenacetin and 30 mg. of caffeine. By comparing results of the two control medications, we have been afforded an opportunity to determine the value of these drugs in our hay fever patients.

Sixty-one unselected patients reporting to the allergy clinics for treatment during the 1950 ragweed season were given 12 of each of the four kinds of tablets (the two antihistamines and two control tablets). Each kind was to be taken for three consecutive days, four tablets a day. Thus the four kinds of tablets were taken during successive three-day periods. All of the tablets were identical in appearance, and the nature of their contents was unknown to the patients.

With the tablets, the patients were given forms on which they were to record daily the following for the three days on each medication:

1. The number of hours per day of absent, mild, moderate or severe symptoms.

2. The daily severity of seven individual hay fever symptoms by degree as absent, mild, moderate, and severe.

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3. Their opinion of the value of each drug on each treatment day by degree as none, slight, moderate, or great.

4. The incidence of side effects for each treatment day.

Not all of the 61 patients completed the course of the four medications. Some patients discontinued the 12 day experiment because of side effects, ineffectiveness, or spontaneous subsidence of their symptoms. Thirty patients took all four, 2 took three, 25 took two, and 4 took only one of the preparations. There was a 100 per cent follow-up of all 61 patients, although many of the forms were returned incomplete.

Because the four medications were passed out and were taken by the patients in completely random order, it was felt that all data, complete and incomplete, must be analyzed, with less prejudice to the results than if the incomplete cases were arbitrarily excluded. Thus we have 30 of the 61 patients reporting on the various sections of the questionnaire for the Phenacetin-caffeine mixture and about the same number for the glucose placebo. This study is confined largely to a comparison of these two groups, a comparison of these groups with the antihistamine-treated patients forming the basis of a separate report.

RESULTS

The total number of hours of absent, mild, moderate, or severe symptoms on both control medications is shown in Table I. Also shown are the number of hours each degree of symptom intensity was experienced during the average patient day. When the hours of absent, and mild symptoms were combined and compared with the hours of moderate and severe, X-square calculations showed no significant difference between the data for the Phenacetin-caffeine compound and the glucose placebo.

TABLE I. TOTAL HOURS OF DIFFERENT DEGREES OF SYMPTOM INTENSITY AND AVERAGE HOURS OF EACH DEGREE PER AVERAGE PATIENT DAY

HOURS OF SYMPTOMS	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	TOTAL	AVE.	TOTAL	AVE.
Absent	668	8.7	539	7.5
Mild	546	7.1	567	7.9
Moderate	393	5.1	423	5.9
Severe	240	3.1	199	2.7
Totals	1847	24.0	1728	24.0

In addition, the hours of symptoms of each treatment day were weighted by multiplying the different degrees of intensity (i.e., severity) by 0, 1, 2, or 3. In this way the total weighted figures (intensity-hours) for each treatment day were obtained. These were averaged for each drug and standard deviations were calculated. For the glucose this was 26.36 ± 1.85. For the Phenacetin-caffeine, 26.69 ± 2.09. Therefore, this analysis also demonstrates lack of statistically significant difference in the effect of the two medications.

Comparing the intensity-hours (calculated by weighting as above) of the 27 patients who took both drugs, we found 11 were better with the Phenacetin and caffeine, 13 were worse, and 3 were the same.

The daily severity of each of seven hay fever symptoms was recorded and the frequency of days of absent, days of mild, days of moderate and days of severe symptoms is shown in Table II. With the percentages to facilitate comparison. The X-square tests for all seven symptoms failed to demonstrate significant differences between the effects of the Phenacetin-caffeine and the glucose.

TABLE II. DISTRIBUTION OF TREATMENT DAYS BY SEVERITY OF SEVEN INDIVIDUAL SYMPTOMS AND OF PER CENTS OF TOTAL TREATMENT DAYS FOR EACH DEGREE OF SEVERITY

DAYS OF SEVERITY	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
Absent	17	22	12	14
Mild	25	32	26	31
Moderate	15	19	31	37
Severe	21	27	15	18
Totals	78	100	84	100

Running Nose

DAYS OF SEVERITY	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
Absent	15	19	14	17
Mild	34	42	24	30
Moderate	20	25	36	44
Severe	11	14	7	9
Totals	80	100	81	100

Stuffy Nose

DAYS OF SEVERITY	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
Absent	36	44	35	43
Mild	31	38	26	32
Moderate	9	11	15	19
Severe	6	7	5	6
Totals	82	100	81	100

Eye Symptoms

SEVERITY DAYS OF	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
Absent	12	15	14	17
Mild	37	45	31	37
Moderate	23	28	29	34
Severe	10	12	10	12
Totals	82	100	84	100

Sneezing

DAYS OF SEVERITY	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
Absent	34	41	26	31
Mild	27	33	26	31
Moderate	13	16	22	26
Severe	8	10	10	12
Totals	82	100	84	100

Itching Nose-Throat

DAYS OF SEVERITY	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
Absent	43	53	43	53
Mild	29	35	24	29
Moderate	9	11	14	17
Severe	1	1	1	1
Totals	82	100	82	100

Fatigue

DAYS OF SEVERITY	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
Absent	66	80	64	75
Mild	7	9	11	13
Moderate	9	11	4	5
Severe	0	0	6	7
Totals	82	100	85	100

Asthmatic Symptoms

TABLE III. DISTRIBUTION OF TREATMENT DAYS BY PATIENTS' OPINIONS OF THE VALUE OF THE DRUGS EACH DAY AND OF PERCENTAGES OF TOTAL TREATMENT DAYS FOR EACH DEGREE OF VALUE

DAYS OF VALUE	PHENACETIN-CAFFEINE		GLUCOSE PLACEBO	
	DAYS	PER CENT	DAYS	PER CENT
None	31	27	29	27
Slight	42	37	38	36
Moderate	28	25	25	23
Great	12	11	15	14
Totals	113	100	107	100

For every day a drug was taken, the patient rated its value in his opinion as none, slight, moderate, or great. These data showing the number of treatment days for each degree of value and their percentages of the total treatment days are shown in Table 3. X-squares on the data indicated no difference between the ascribed values of the two drugs.

Of 44 patients on the glucose, 34 per cent experienced side effects. Of 34 patients on the Phenacetin-caffeine, 38 per cent reported side effects. These were similar for each drug, consisting mainly of drowsiness, headache, and nausea. Noteworthy are two patients who had to discontinue the glucose because of the nausea and vomiting they felt it produced.

In the study for which the Phenacetin-caffeine and glucose were used as controls, the antihistamine Thenylpyramine Hydrochloride (30 mg. 4 times daily) was compared to a combination of the same antihistamine plus Phenacetin-caffeine in the control dosage. Comparison of results with the antihistamine alone and the antihistamine plus the Phenacetin-caffeine should be of value in determining the therapeutic effectiveness of Phenacetin and caffeine (in the dosages employed) in hay fever.

Total hours and intensity of symptoms of hay fever for the two medications are shown in Table IV. When hours of absent and mild symptoms are combined and compared with hours of moderate and severe, X-square calculations show no significant difference for the Thenylpyramine plus Phenacetin-caffeine as compared to the Thenylpyramine alone.

Weighting the figures and obtaining intensity-hours of symptoms with their standard deviations as previously described gave 23.18 ± 1.99 for the antihis-

TABLE IV. TOTAL HOURS OF DIFFERENT DEGREES OF SYMPTOM INTENSITY AND AVERAGE HOURS OF EACH DEGREE PER AVERAGE PATIENT DAY FOR ANTIHISTAMINE ALONE AND WITH PHENACETIN AND CAFFEINE

HOURS OF SYMPTOMS	THENYLPYRAMINE HYDROCHLORIDE		THENYLPYRAMINE HYDROCHLORIDE PLUS PHENAC-CAFFE	
	TOTAL	AVE.	TOTAL	AVE.
Absent	900	11.0	916	10.6
Mild	446	5.5	540	6.3
Moderate	410	5.1	386	4.4
Severe	191	2.4	236	2.7
Totals	1947	24.0	2078	24.0

tamine-Phenacetin-caffeine combination and 21.23 ± 2.01 for the antihistamine alone. These results again do not differ significantly.

Similarly, analysis of individual symptoms of hay fever showed no significant differences following treatment with these two medications; and this was also true of the patients' opinions of the value of two medications.

In short, the use of Phenacetin and caffeine with the antihistaminic did not produce therapeutic results significantly different from those obtained with the antihistamine alone.

SUMMARY AND CONCLUSIONS

Treatment of a group of hay fever sufferers with a mixture containing 200 mg. Phenacetin and 30 mg. caffeine given four times a day for three days was compared with results obtained with a glucose placebo taken in the same way by a similar group. There was essentially no difference between the two groups with regard to effectiveness in relieving the hay fever in general and in relief of the following individual symptoms: running nose, nasal stuffiness, eye symptoms, sneezing, itching nose and throat, fatigue, and asthma. Opinion of the patients as to value and side effects gave almost identical data for the two medications. Statistical analysis of the data showed no significant difference between the effects of the Phenacetin-caffeine and the glucose placebo. Similarly, the use of an antihistamine (Thenylpyramine Hydrochloride, 30 mg.) in combination with Phenacetin and caffeine did not produce significantly different therapeutic results than did the antihistamine alone.

MOLDS IN HOUSE DUST, FURNITURE STUFFING, AND IN THE AIR WITHIN HOMES

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HOUSE dust and furniture stuffing have long been known to contain allergenic substances,[4, 5] and both are known to harbor a variety of molds.[3, 6, 7, 8] There are, however, almost no quantitative data on the numbers and kinds of molds present in these heterogeneous materials; nor, in most of the studies reported in the literature, have culture media been used that had been selected or designed especially to disclose those species of molds that might be expected to predominate.

The aims of the work here reported were (1) to determine the number and kinds of molds present in house dust, and in new and used furniture stuffing, using techniques and media that had proved to be of value in assaying the molds in other but somewhat similar substrates. (2) To determine whether bacteria were present in any numbers in house dust or furniture stuffing. (3) To compare the numbers and kinds of molds present in the air within homes with those encountered in house dust or furniture stuffing.

MATERIALS AND METHODS

Collection of Samples.—Dust samples were collected from vacuum cleaners in 76 homes in and near St. Paul and Minneapolis, Minnesota, during the winter months of 1950 and 1951 to 1952. New and used furniture stuffing was obtained from several furniture repair shops in the two cities. All of the samples were placed in sterile paper bags, brought to the laboratory, and stored dry until assayed for microflora.

Mold Count.—Portions of 0.5 or 1.0 Gm. were weighed on a sterile filter paper on a torsion balance sensitive to 0.01 Gm., placed in 50 or 100 ml. of a 0.3 per cent sterile solution of agar in water, shaken on a mechanical shaker at approximately 400 strokes per minute for ½ hour. Subsequent dilutions were made by transferring 5 ml. aliquots to successive portions of sterile dilute agar solution, and two or more dilutions were cultured in malt-salt agar. The weak agar solution was used to suspend the materials, and also to make the successive dilutions, because tests on other materials had shown that mold spores were likely to be much more uniformly distributed in such suspensions than in water; it gave more consistent and uniform results, presumably because it prevented mold spores from settling out.

Several different media were tested in preliminary work. As a result of these tests, and of experience with mold assays of a variety of other substances, malt-salt agar appeared to be preferable to any of the others used. This medium

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has rather consistently yielded a larger number of colonies of a greater variety of fungi in assays of house dust, in rather extensive air exposures made by us over the past two years, in the culturing of flour[1] and various kinds of stored seeds[2] than any other medium tested. It seems particularly well suited to bringing out those fungi which grow where no free water is present. Such fungi might reasonably be expected to predominate in various places within the average home. Malt-salt agar is selective to the extent that few bacteria and yeasts are able to develop on it. Unquestionably it eliminates certain molds also, but in our experience it has yielded a larger number of colonies of a greater variety of fungi than any of the numerous other media tested, and this seemed sufficient justification for using it throughout the study.

Malt-salt agar consists of 20 Gm. of malt-extract, 20 Gm. of agar, 75 Gm. of sodium chloride (either technical grade, or rock salt have proved satisfactory; table salt has been unsatisfactory), and 1 L. of water. The materials are dissolved in the water by heating, autoclaved at 15 pounds steam pressure for 20 to 30 minutes, and stored until used.

The sampling of house dust for bacteria was similar to the above, except that beef-peptone agar was used as a culture medium. It is recognized that many kinds of bacteria may have been present which did not appear on this one medium.

The culture dishes containing the diluted material were incubated in the laboratory at a temperature of 20 to 23° C. The colonies of molds were counted after five days, and identified shortly thereafter.

Molds in the Air Within Homes.—Duplicate culture dishes containing malt-salt agar were exposed at several different times of the day in each of 15 different homes in St. Paul, during the spring and early summer of 1950 and 1951. At the same time, dishes were exposed out of doors at the same locations. All exposures were for 12 minutes. After having been exposed to the air, the dishes were covered, wrapped in sterile paper, brought to the laboratory, and incubated until the colonies could be counted and identified.

RESULTS

Number of Molds in House Dust.—A summary of the number of molds isolated from house dust samples from 76 homes is presented in Table I.

TABLE I. NUMBER OF MOLDS IN HOUSE DUST

MOLDS PER GM.	NO. OF SAMPLES
Less than 10,000	4
10,000-100,000	51
101,000-500,000	15
501,000-1,000,000	3
More than 1,000,000	3

Range in mold count: Lowest—6,000/g. Highest—3,200,000/g.
Average mold count of 76 samples—179,966/g.

Number of Bacteria in House Dust.—The numbers of bacteria in samples of house dust collected from 14 homes in the winter of 1952 are summarized in Table II.

TABLE II. NUMBER OF BACTERIA IN HOUSE DUST

Number of samples—14	
Number per Gm.:	Average—10,700,000
	Range—1,144,000-20,000,000

Numbers of Molds and Bacteria in Furniture Stuffing.—These data, including results from various types of foam rubber, are summarized in Table III.

Mold Spores in the Air Within Homes.—The data from seven different homes are presented graphically in Fig. 1.

DISCUSSION

House dust contains large numbers of a considerable variety of molds. The greater proportion of the molds in the samples here reported upon were species of Penicillium and Aspergillus, principally those able to grow in a great variety of materials whose moisture content is in equilibrium with a relative humidity of 70 to 90 per cent. Although it was not proved directly, the evidence suggests that most of the molds in houst dust are growing within the house itself, either in dust or in the materials that contribute to the dust.

New furniture stuffing is by no means free of molds, and used furniture stuffing may have very high counts, principally of the species of Penicillium and Aspergillus as were encountered in house dust. It seems highly probable that the increase in mold count in used furniture stuffing is primarily from molds actually growing and sporulating in and on the stuffing.

None of the samples of new foam rubber were free of molds, although all had a relatively low count. The mold count of used foam rubber was higher than that of new. Samples of new foam rubber incubated in moist chambers were gradually invaded by molds, and various of these molds fruited on the surface and within air bubbles beneath the surface of the foam rubber.

Numbers of bacteria in house dust and in furniture stuffing far exceeded the mold count; it would seem desirable to explore bacteria in these materials fairly throughly as possible allergens. Very few bacteria were found in either new or used foam rubber of any of the several types and brands tested.

In the plates exposed indoors, the total mold counts were, in general, somewhat lower than exposures outside, but the kinds of molds in the two locations differed. In general, Penicillium and Aspergillus predominated in the indoor air, while Alternaria and Cladosporium predominated outside. The number of mold spores caught in the air indoors fluctuated sharply during a single day, apparently varying according to the amount of physical activity within the home at or just prior to the time the culture dishes were exposed. Some houses with a low mold count in the air, as determined by plate exposures, had a relatively

TABLE III. MOLDS AND BACTERIA IN NEW FURNITURE STUFFING

MATERIAL	MOLDS PER GM.	BACTERIA PER GM.
Cotton	11,000	1,680,000
Kapok	4,000	22,000
Foam rubber	1,000	60

high mold count in the dust taken at the same time from vacuum sweepers: some with a high mold count in the air had a relatively low count in the dust from vacuum sweepers. In some cases, one species of mold would predominate in the dust taken from a vacuum sweeper, and another type in the air. Evidently studies more detailed and thorough than those reported here will be necessary to determine accurately the range in kinds and numbers of molds or other microflora to which an occupant of a given home might be exposed at a given time. Each house is a separate entity.

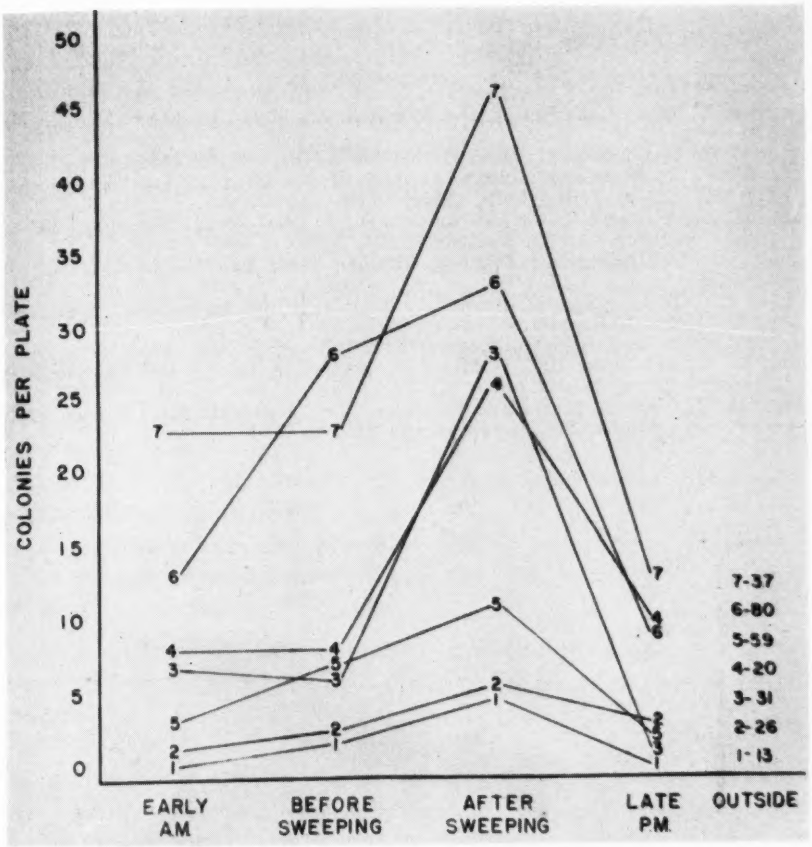

Fig. 1.—Average number of colonies obtained from within seven different homes at four times during the day, and from the outside air.

SUMMARY

1. The mold colonies isolated from 76 samples of house dust collected in homes in and near St. Paul and Minneapolis, Minnesota, in the winters of 1950 and 1951 to 1952 varied from less than 10,000 per gram to more than 3,000,000 per gram, with an average of 179,966 mold colonies per gram.

2. The number of bacteria from 14 samples of house dust ranged from 1,144,000 to 20,000,000 per gram, and averaged 10,700,000 per gram.

3. Molds and bacteria were present in some numbers in the samples of new furniture stuffing, and were present in considerably greater numbers in some used materials.

4. Foam rubber appears to be somewhat susceptible to invasion by molds, though to a lesser degree than certain fibers. Bacteria do not appear to be present in any considerable numbers in either new or used foam rubber.

5. The molds present in the air within homes may differ in both kind and numbers from those in the outdoor air at the same time. The number of viable mold spores (or other inoculum) in the air within homes may fluctuate sharply during a single day, apparently increasing when the amount of physical activity within the home increases.

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A STUDY OF ANTIBIOSIS BETWEEN WIND-BORNE MOLDS AND INSECT LARVAE FROM WIND-BORNE EGGS

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A SURVEY was made by the writer in 1950 on molds in the Savannah area. This work was done partly in cooperation with The American Academy of Allergy in its nationwide study on molds as possible causative agents of inhalant allergy. In the report of the survey[1] it was stated that occasionally insect larvae would appear in the culture dishes which had been exposed for mold growth; and that each time this occurred, mold colonies did not develop fully, and finally disappeared completely.

This paper is reporting results of further study on insect larvae, which arose from wind-borne eggs, preventing or disturbing mold growth on a culture medium, and other antibiotic activities noted while making the study.

Some of the questions to which answers were sought in this study were:

1. What insect, or insects, whose eggs are wind borne, will develop into larvae that will prevent or disturb mold growth on a culture medium?
2. Will the larvae prevent mold growth on a culture medium if they are present on the medium at the time of exposure in the outside atmosphere?
3. How is the mold growth affected if egg deposits are made in the culture dish immediately following exposure?
4. Are the larvae capable of destroying mold colonies after 3 or 4 days of development?
5. Are there any variations in the resisting powers of the different molds to the destructive actions of the larvae?
6. Do the molds have any effects on the life span of the mature insect?
7. Do the larvae-mold growths cause any change in the hydrogenion concentration of the medium?

No special equipment and material were needed in making this study. The equipment and material used are ordinarily found in a general bacteriology laboratory.

METHODS

The method of collecting the wind-borne insect eggs was identically the same as used in collecting wind-borne spores for mold colonies. The plates were observed carefully to guard against any insect from making direct contact with the plates during exposure. After the larvae became visible to the naked eye, the top of the Petri dish was removed, and the dish was transferred to a liter beaker covered with a piece of finely woven cloth. This provided ample space and air for complete development of the larvae into mature organisms. After development had been completed, the insects were then identified and kept for future experimental work with the molds.

Two hundred fifty-seven plates were used in this phase of the study. They were exposed at different times of the day, and in various climatic conditions.

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Controlled laboratory experiments were performed with Petri dishes in the following manner. Six Petri dishes were used in this experiment. Nonabsorbent cotton was placed around the rims of 5 of the dishes, and one remained without cotton. Five of these dishes were exposed to the outside atmosphere for 12 minutes, at the same time, and in the same location. At the end of this period, all of the dishes were closed.

Dish No. 1 was used as a control to show types and quantity of molds present for that period.

Dish No. 2 was a control to show development of mold colonies that had access to increased amount of air filtered through nonabsorbent cotton.

Dish No. 3 contained the insects (*Drosophila melanogaster*) that developed from wind-borne eggs. Twenty of them were placed in the dish immediately following the period of exposure, and remained for 24 hours for egg deposits.

Dish No. 4 contained the insects the same as above, but they remained in the dish throughout the 10 day period.

Dish No. 5 had larvae in it at the time of exposure.

Dish No. 6 contained 3-day-old mold colonies before the insects were placed in it.

All of the dishes were incubated at room temperature, and checks were made on their development over a period of 10 days.

Other experiments were made with individual molds and the insects. The molds used were: *Aspergillus niger,* Mucor, *Penicillium camemberti,* Alternaria, Hormodendrum, Curvularia, Fusarium, *Aspergillus clavatus,* Monilia *Penicillium glaucum,* Macrosporium, and Rhizopus. Sterile casein hydrolysate slants were used in these tests. Two slants were used for each mold, and they were inoculated at the same time. In one of the tubes, 12 of the insects were placed for a period of 24 hours for egg deposits. At the end of this period, the insects were removed from the tube, and both tubes incubated at room temperature. The other tube was the control. Other experiments were performed in the same way only the mature insects remained in the tubes. Observations were made periodically over a period of 10 days on the mold growth.

Tests were also made to determine any change in the hydrogen-ion concentration of the medium caused by these organisms. The "set up" was similar to the preceding experiment, and after 10 days of incubation, chemical analyses were made on the slants using a Beckman, Glass Electrode pH meter.

Eight tests were performed on each of the experiments, and all data were carefully recorded.

RESULTS

From my observations on the 257 culture dishes exposed in the outside atmosphere, 18, or about 7 per cent of them, became contaminated with insect larvae. The larvae in all of the dishes developed into mature insects, which have been identified as *D. melanogaster* (a species of fruit fly). All plates were pure cultures of larvae of the *D. melanogaster.* The first dish that became con-

taminated with these larvae was noted in May, and the last one in September. The greatest contamination occurred from the middle of June to the last of August.

When the *D. melanogaster* were permitted to deposit eggs in the culture dishes immediately following exposure in the outside atmosphere for a period of 12 minutes, mold colonies developed to the stage that they could be seen

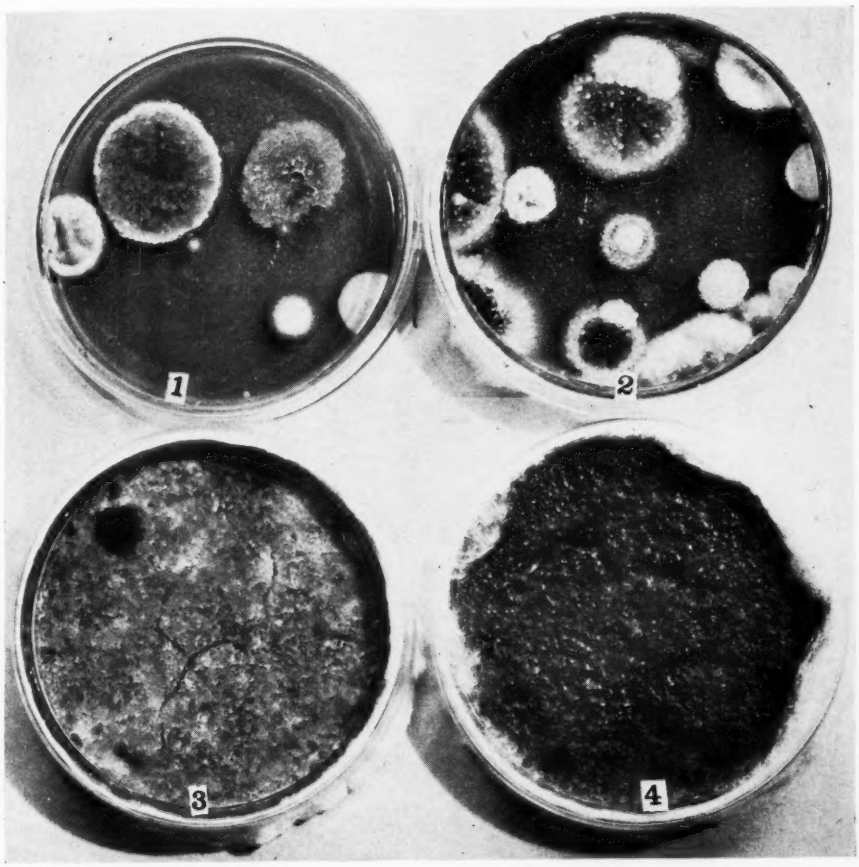

Fig. 1.—Cultures of wind borne molds and *Drosophila melanogaster* larvae at the end of 5 days of incubation at room temperature. (1) Control. Normal mold colony growth (identical with the Mold Survey Study); (2) Development of mold colonies that had increased amount of air filtered through nonabsorbent cotton; (3) Egg deposits were made immediately following exposure in the outside atmosphere. Increased supply of air was identical with No. 2; (4) Five-day-old larvae were present in the culture dish at the time of exposure. Air supply was the same as in Numbers 2 and 3.

with the naked eye, or to the time when the larvae became visible with the aid of a magnifying glass. After this stage of development, the mold colonies began showing signs of disintegration, and finally disappeared completely by the end of the eighth day.

Mold colonies did not develop to the extent that they could be seen with the unaided eye if the flies were allowed to deposit eggs in the dish 24 hours prior to outside atmospheric exposure.

TABLE I. TWELVE DIFFERENT MOLD GROWTHS CONTAMINATED WITH DROSOPHILA MELANOGASTER FLIES

NAME OF ORGANISM	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY	9TH DAY	10TH
Aspergillus niger	SG	MG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	SG	MG	AG	AG FD	AG	AG	AG	AG	AG	AG
Mucor	SG	MG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	SG	SG	GPD	GPD	GD WL	NG	NG	NG	NG	FD NG
Penicillium camemberti	SG	MG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	MG	MG	GPD WL	GPD WL	FD GPD WL	GPD WL	GPD WL	GPD
Alternaria	NG	SG	MG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	GPD WL	GPD WL	GPD WL	GPD WL	FD GPD WL	GPD WL	GPD WL	GPD WL
Hormodendrum	NG	SG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	GPD WL	GPD WL	GPD WL	GPD WL	GPD WL	FD GPD WL	GPD WL	GPD WL
Curvularia	NG	SG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	GPD WL	GPD WL	GPD WL	GPD WL	GPD WL	GD WL	FD NG WL	NG WL

NAME OF ORGANISM	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY	9TH DAY	10TH
Fusarium	NG	MG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	GPD WL	GPD WL	GPD WL	GPD WL	GPD WL	FD GPD WL	GPD WL	GPD WL
Aspergillus clavatus	NG	MG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	MG	GPD NL	GPD WL	GPD WL	GD WL FD	NG	NG	NG	NG
Monilia	NG	MG	MG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	GPD NL	GPD WL	GPD WL	GPD WL FD	GPD WL	GPD WL	GD	NG
Penicillium glaucum	SG	MG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	SG	MG	AG	AG FD	AG	AG	AG	AG	AG	AG
Macrosporium	NG	MG	AG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	GPD	GPD	GPD	GPD	GD WL	NG	NG	NG
Rhizopus	NG	SG	MG	AG	AG	AG	AG	AG	AG	AG
C										
E	NG	SG	GPD NL	GPD WL	GPD WL	FD GPD WL	GD WL	NG	NG	NG

Key: C = control tube
E = experimental (tube with flies and fungi)
NG = no growth
SG = scanty growth
MG = moderate growth
AG = abundant growth
GPD = growth partly destroyed
GD = growth destroyed
NL = no larvae
WL = with larvae
FD = flies dead

Mold colonies did not develop to the stage that they could be detected with optical instruments when exposures were made with dishes that contained larvae.

Three-day-old colonies were completely destroyed by larvae at the end of the eighth day of incubation.

Some of these observations can be seen in Figs. 1 and 2.

The results of the experiments with individual molds and the flies showed great variations in: (1) amount of growth of the molds in the culture tubes,

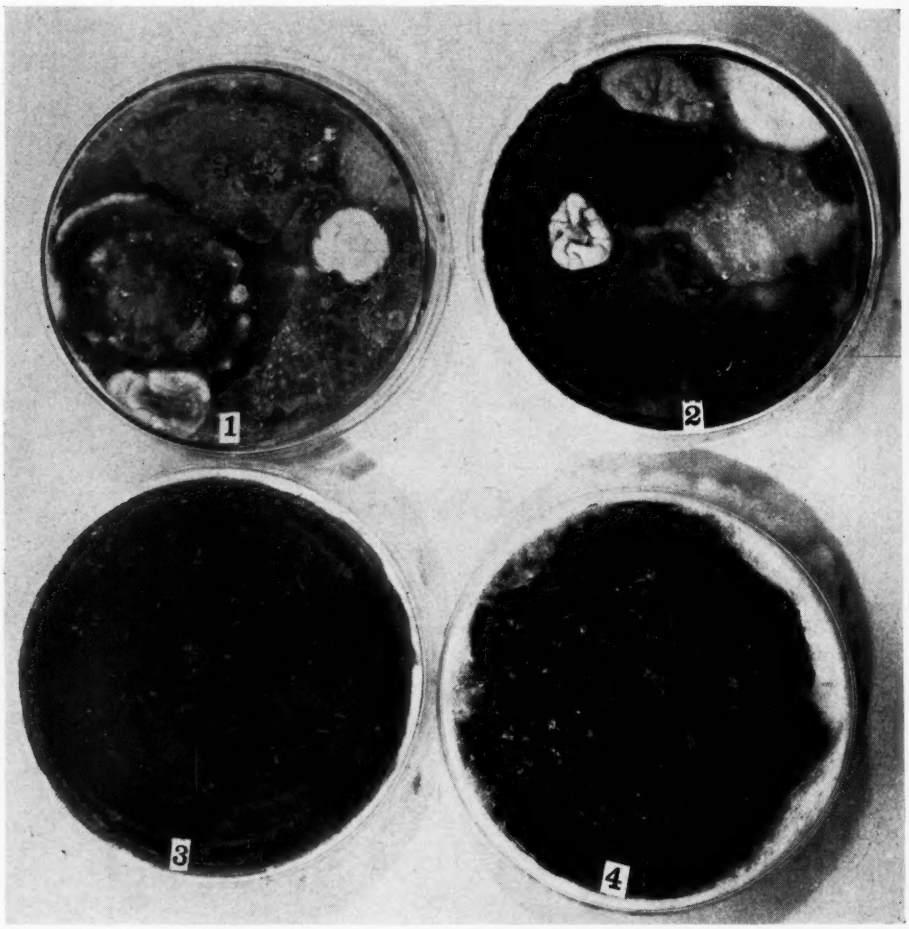

Fig. 2.—Same cultures as in Fig. 1 after 10 days of incubation.

(2) the length of time that the flies were able to live among the various molds, and (3) larvae development.

The average life span of the flies grown in tubes of the sterile culture medium was 9 days.

The results of these observations are illustrated in Table I and Fig. 3.

The pH of the medium used in these experiments was 6.8. After 10 days of incubation, the organisms changed the pH of the medium as indicated in Table II.

Fig. 3.—Twelve different mold growths contaminated with *Drosophila melanogaster* flies at the end of 5 days' incubation at room temperature.

CONTROLS (molds only)

Tube No. 1.	Aspergillus niger	Tube No. 13.	Fusarium
Tube No. 3.	Mucor	Tube No. 15.	Aspergillus clavatus
Tube No. 5.	Penicillium camemberti	Tube No. 17.	Monilia
Tube No. 7.	Alternaria	Tube No. 19.	Penicillium glaucum
Tube No. 9.	Hormodendrum	Tube No. 21.	Macrosporium
Tube No. 11.	Curvularia	Tube No. 23.	Rhizopus

EXPERIMENTS (molds and flies)

No. 2.	Aspergillus niger	No. 14.	Fusarium
No. 4.	Mucor	No. 16.	Aspergillus clavatus
No. 6.	Penicillium camemberti	No. 18.	Monilia
No. 8.	Alternaria	No. 20.	Penicillium glaucum
No. 10.	Hormodendrum	No. 22.	Macrosporium
No. 12.	Curvularia	No. 24.	Rhizopus

TABLE II

WITH MOLD ONLY	pH	WITH MOLD AND LARVAE	pH
Aspergillus niger	7.6	Aspergillus niger	4.1
Mucor	6.8	Mucor	5.2
Penicillium camemberti	7.5	Penicillium camemberti	8.7
Alternaria	8.4	Alternaria	5.7
Hormodendrum	5.7	Hormodendrum	8.2
Curvularia	8.1	Curvularia	5.7
Fusarium	9.3	Fusarium	5.7
Aspergillus clavatus	6.0	Aspergillus clavatus	7.8
Monilia	6.2	Monilia	5.8
Penicillium glaucum	5.8	Penicillium glaucum	6.0
Macrosporium	7.4	Macrosporium	5.8
Rhizopus	6.4	Rhizopus	5.4
With larvae only	4.9		

These observations might be of some use to those who are primarily interested in the study of causative agents of inhalant allergy. The fact that approximately 7 per cent of the total number of Petri dishes exposed in the outside atmosphere became contaminated with *D. melanogaster* eggs that were wind borne, and that these eggs and their larvae do affect the growth of certain molds, should be worthy of further study, as is also the possibility that these eggs might be a cause of certain respiratory allergies.

The variations in hydrogen-ion concentration caused by the molds, larvae, and mold-larvae cultures may be considered as an initial step for further study by the chemist, which might be of use in combating certain respiratory allergies.

SUMMARY

1. *D. melanogaster* eggs are wind borne, and they develop larvae that will prevent, destroy, or retard the growth of certain species of molds on a culture medium.

2. The larvae prevent mold growth on a culture medium if they are present on the medium at the time of exposure in the outside atmosphere.

3. If egg deposits are made in the culture dishes immediately following exposure in the outside atmosphere, mold colonies will develop very slowly. However, they will disappear soon after the larvae make their appearance.

4. The larvae are capable of destroying some mold colonies 4 days old.

5. There are variations in the resisting powers of the different molds studied. Some prevent development of larvae completely.

6. Several of the molds have definite effects on the life span of the mature flies. Some kill the flies as early as the fourth day.

7. The larvae, molds, and larvae-mold growths cause changes in the hydrogen-ion concentration of the medium.

The author thanks William H. Bowens, Savannah State College, Savannah, Ga. for the photographic plates used in this paper.

REFERENCE

1. Griffith, B. T.: Mycological Studies in the Savannah Area 1950, J. ALLERGY **22**: 465, 1951.

Editorial

FATAL ALLERGIC REACTIONS TO PENICILLIN

Waldbott, in 1948, reported a case of sudden death with the manifestations of anaphylactic shock occurring after an injection of penicillin. Despite this isolated report, the great majority of physicians have continued to use penicillin freely in treatment of patients with or without a background of allergic disease. At a recent meeting of the New York Allergy Society, the possible danger of such promiscuous use of penicillin was brought out sharply. Dr. Sheppard Siegal reported three cases of severe immediate reactions of the anaphylactic type following injections of penicillin, one of which proved fatal. The ensuing discussion revealed that the sixty members present had personal knowledge of three other fatal anaphylactic reactions to penicillin, not yet reported in medical journals, and a large number of alarming reactions of the same immediate type in which recovery occurred. The citation of so many cases in a meeting of this size certainly suggests that such immediate reactions, while far less common than the familiar delayed urticarial reactions, are more frequent than the medical literature indicates.

A great majority of the patients cited as having severe immediate reactions of this type gave a past or family history of allergic disease of the hereditary or atopic type; many suffered from asthma. All had received numerous injections of either crystalline or procaine penicillin at irregular intervals, usually for the treatment of recurrent respiratory infections. In some instances, previous injections had caused mild but definite immediate reactions, in others the severe or fatal reaction occurred suddenly after previous doses had been well tolerated. In none of the cases cited had the development of sensitization to penicillin been accompanied by a delayed urticarial reaction similar to serum sickness. All of the fatal reactions followed intramuscular injections, but very severe and equally prompt reactions were reported after instillation of penicillin into the maxillary sinus, inhalation therapy, and oral administration of the drug as tablets. The reactions following the use of crystalline and procaine penicillin were essentially similar, and in no case was there any evidence that the procaine moiety caused the sensitivity. In no instance was there clinical evidence, nor in the two cases coming to autopsy, anatomical evidence, of embolism or other nonallergic cause for the reaction.

Several of the patients who recovered from severe anaphylactic reactions subsequently showed immediate wheal reactions to scratch or intracutaneous tests with penicillin, and in some instances the Prausnitz-Küstner reaction was demonstrable. Intracutaneous tests on some of these patients were followed by severe constitutional reactions. These skin reactions are in striking con-

trast to the negative or questionable results noted in patients with the delayed urticarial type of reaction to penicillin, but consistent with the skin reactions elicited by thiamin, quinine, and occasionally sulfonamides in patients suffering similar anaphylactic reactions from those agents.

While these anaphylactic reactions to penicillin are relatively rare in proportion to the tremendous number of patients treated with the drug, their occurrence, in addition to that far more frequent but less serious delayed urticarial reactions, suggests careful consideration of the repeated use of the drug in patients with allergic diseases. Certainly the occurrence of even a mild immediate allergic reaction after one injection should cause hesitation in repeating the treatment. In most cases one of the other antibiotic agents might be substituted. Use of the arm or thigh rather than the buttocks as the site of injection should lessen the danger by permitting the use of a tourniquet if necessary. The use of penicillin O in patients who had previously received penicillin G might lessen the chance of reaction to a particular dose, but there is no assurance that penicillin O is not an equally active sensitizer if used repeatedly. The combination of penicillin with antihistamine drugs has also been suggested, but the rapidity and severity of the reactions cited raise serious doubt as to whether any reasonable dose of antihistaminic injected simultaneously would have a sufficiently prompt and potent effect to prevent a serious outcome. Since the danger of this type of reaction is becoming apparent only after millions of injections of penicillin have been given over a period of ten years, favorable results in a few hundred cases treated with penicillin-antihistamine combinations have no statistical significance.

The positive reactions to skin tests noted in several of the patients suggest a possible aid in foreseeing and avoiding these reactions. Further studies will be needed to establish the actual value of such tests and to determine a technique of testing which will be sufficiently sensitive to yield reliable results without in itself producing dangerous reactions. Present indications are that scratch tests with solutions containing 10,000 to 50,000 units of crystalline penicillin per millimeter or intracutaneous tests with 0.01 to 0.02 ml. of solutions containing 100 to 1,000 units per milliliter might serve the purpose. Suspensions of procaine penicillin in oil or water are apparently not useful for skin tests.

RELATIONSHIP BETWEEN INOCULATIONS AND POLIOMYELITIS

(Reprinted in Part From the Public Health Reports May, 1952, Vol. 67)

The possible relationship of various types of inoculations and poliomyelitis has been a topic of much discussion and a number of papers since the 1950 reports from England. Last fall the State and Territorial Health Officers Association asked the Public Health Service of the Federal Security Agency to sponsor a study on the question and issue a clarifying statement. Subsequently, the Public Health Service, on March 14, 1952, sponsored a meeting of 42 poliomyelitis investigators, epidemiologists, pediatricians, allergists, and health officers. The National Foundation for Infantile Paralysis helped plan and participated in the conference.

The conference voted unanimously in favor of the conclusions contained in the following statement which has been accepted by the Public Health Service and is being transmitted to official health agencies, to the medical profession and to the general public.

There is no definite evidence that an increase in the number of cases of poliomyelitis has occurred as a result of injections of vaccines, drugs, and other medicinal agents. There is evidence that injections for the prevention of diphtheria, whooping cough, and possibly tetanus, when given during an epidemic of poliomyelitis, may, on rare occasions, localize the paralysis in the inoculated arm or leg. There is no satisfactory evidence that other types of injections have any effect on the localization, frequency, or severity of poliomyelitic paralysis.

Certainly no parent should object and no physician should hesitate to administer a needed antibiotic, drug, or other injection for treatment of disease at any time. In the final analysis the decision as to when an immunizing or therapeutic injection shall be given to an individual patient must rest with the physician.

Erratum

In the President's Address, American Academy of Allergy section of the JOURNAL (May issue, page 281), the name of the author, Dr. Horace S. Baldwin, retiring president of the American Academy of Allergy, was omitted.

Book Review

Annual Report on Stress, by Hans Selye, M.D., Ph.D., Montreal, Acta Inc., 1951, 511 pages, $10.00

One has only to peruse the appendix of 133 pages, listing 3,000 references, most of which have been published since 1949, to realize the need for frequent reviews of the enormous and rapidly growing literature on the subject of stress in relation to the pituitary and adrenal hormones. Workers having occasion to follow this mass of literature will find this volume a valuable index, regardless of their opinion of the author's interpretations.

In reviewing the contributions to the subject, the author is naturally biased by their bearing on his well-known concept of the General Adaptation Syndrome, and the application of new facts to the development of this concept is the chief theme of the book. The present work is intended purely as a sequel to the author's previous volume *Stress*, published by the same house in 1950 and duplication of material previously presented is avoided. Therefore, readers who are not already thoroughly familiar with the General Adaptation Syndrome would be wise to seek their introduction to the subject in the previous volume if they are to understand the present one.

Consideration is given to criticisms of the Adaptation Syndrome based on the therapeutic benefits of cortisone and corticotrophin in diseases classed as Diseases of Adaptation, and the concept of balance between antagonistic hormones of the pituitary gland (sometotrophic and adrenocorticotrophic) and of the adrenal cortex (zheco-corticoids and mineralocorticoids) is developed. It is apparent that much more experimental evidence is needed before this relationship can be clearly established. Despite the activity of research in experimental endocrinology, the empirical application of these hormones in clinical therapeutics has far outrun its theoretical basis.

Announcement

RAGWEED POLLEN SURVEY

Copies of the 1952 edition of the booklet containing all available ragweed pollen indexes for communities in North America are available for distribution on request at the office of the Executive Secretary of the American Academy of Allergy.

The Pollen Committee suggested that these booklets be made available to students in medical schools. Copies for this purpose will be sent as long as the supply lasts.

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